

# Genome-wide analysis of ATP-binding cassette transporters sheds light on the genes related to bioactive metabolite transportation in *Salvia miltiorrhiza*

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## Research article

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# Abstract

**Background:** ATP-binding cassette (ABC) transporters have been found in plants and play important roles in metabolic transport in cells, which affect the subcellular compartmentalisation and tissue distribution of these compounds in particular. The dry roots and rhizomes of *Salvia miltiorrhiza* Bunge, a widely used medicinal plant, known as Danshen in traditional Chinese medicine, contain biologically active secondary metabolites of tanshinone and salvianolic acid. Given an assembled and annotated genome and a set of transcriptome data of *S. miltiorrhiza*, we analysed and identified the candidate genes that likely involved in the bioactive metabolite transportation of this medicinal plant, starting with the members of the ABC transporter family.

**Results:** A total of 114 genes encoding ABC transporters were identified in the genome of *S. miltiorrhiza*. All the ABC genes were divided into eight subfamilies, including 3ABCA, 31ABCB, 14ABCC, 2ABCD, 1ABCE, 7ABCF, 46ABCG and 10 ABCI. Gene expressional profiling analysis revealed the tissue-specific expression profiles of these ABC transporters, particularly the highly expressed transporters in the roots of *S. miltiorrhiza*, which might be involved in transporting bioactive compounds of this medicinal plant. Notably, 18 genes that were highly expressed in the roots of *S. miltiorrhiza* were selected and verified by quantitative reverse transcription polymerase chain reaction to determine the co-expression profiling of these candidate genes with the key enzyme genes involved in the tanshinone and salvianolic acid biosynthetic pathways. Finally, three ABC genes (SMil\_00020022, SMil\_00010949, SMil\_00027268) and one gene (SMil\_00016361) might be involved in tanshinone and salvianolic acid transport in cells, respectively. In addition, the biological functions of *S. miltiorrhiza* ABC transporters were predicted on the basis of the phylogenetic analysis and expressional profile exploration, including the transportation of lipids, hormones, ions and primary metabolites.

**Conclusions:** Our systematic analyses on the ABC transporters in *S. miltiorrhiza* provided information on ABC proteins for the first time and revealed the candidate transporters involved in the bioactive compound transportation of this medicinal plant. The genome-wide identification, transcriptome profile analysis and their phylogenetic relationships would provide a new perspective on the function of ABC transporters in *S. miltiorrhiza*.

## Background

The ATP-binding cassette (ABC) transporters constitute one of the gene families in all living organisms ranging from prokaryotes to eukaryotes, most of which are involved in a wide range of biological processes, and play key roles in the transmembrane transport of metabolites across biological membranes by hydrolysing ATP in plant cells [1]. In most cases, the core unit of functional ABC transporters usually consist of a combination of two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). The TMDs, which typically contain several (usually four to six) transmembrane hydrophobic alpha-helices, form the membrane-spanning pore for substrate recognition and solute movement across the phospholipid bilayer. The two NBDs couple ATP hydrolysis and ADP

release to provide the driving force for transport. This domain contains several key conserved motifs, namely, Walker A (GX<sub>4</sub>GK(ST)), Walker B ((RK)X<sub>3</sub>GX<sub>3</sub>L(hydrophobic)<sub>3</sub>), ABC signature, Q-loop, D-loop and H-loop [2–4]. In general, ‘full-sized’ ABC proteins comprise two couples of TMD–NBD, which function as transporters, whereas ‘half-sized’ ABC proteins have one TMD–NBD that must form homo- or heterodimers to become transporters [3].

Genome analyses of model plants (e.g. *Arabidopsis* and rice) show that the plant genome contains a large number of ABC transporters, which exceed the ABC genes in the genomes of animals and other eukaryotes [2]. The increase of ABC genes has significantly improved the ability of plants to adapt to various environmental stresses [3]. ABC transporters are usually located on plant cell plasma membranes, vacuole membranes and other organelle membranes and regulate the absorption and efflux of specific substances across the membrane in plant cells, such as secondary metabolites, sugars, amino acids, plant hormones, lipids and metal ions [5, 6]. The various biochemical and physiological functions of ABC proteins are relevant for the transport of diverse substrates, disease resistance and detoxification, which are necessary for maintaining plant life [3].

The subfamily classification of plant ABC transporters is performed according to the subfamily nomenclature proposed by the Human Genome Organization [7]. This nomenclature is based on the phylogenetic correlation of the NBD amino acid sequence. On the basis of aligning amino acid sequences of NBDs and performing phylogenetic analyses, the eukaryotic ABC transporter family is divided into eight subfamilies, namely, ABCA, ABCB, ABCC, ABCD, ADCE, ABCF, ABCG and ABCH [8]. However, no ABCH subfamily is found in plants; while, ABCH is replaced by ABCI, which exists in plants but is absent in animals. The division of these subfamilies is based on the phylogenetic relationship of the amino acid sequence of NBD and also largely supported by domain organisation (the order of domains in the ABC protein), although some examples of subfamilies include both full-sized and half-sized transporters [7]. The plant ABC subfamily is also named after its human or microbial prototype, for example, ‘pleiotropic drug resistance’ (PDR) and ‘multidrug resistance-related protein’ (MRP) [2]. In plants, the best-identified subfamilies are the multidrug resistance (MDR), MRP, PDR and white–brown complex homologue (WBC) subfamilies [6]. The *Arabidopsis* ABC protein superfamily consists of the full-sized transporters, half-sized transporters and soluble proteins [6]. The full-sized transporters include the MDRs, MRPs, PDRs and ABC one homologues (AOHs). The half-sized transporters include the peroxisomal membrane proteins (PMPs), WBCs, ABC two homologues (ATHs), ABC transporter of the mitochondrions (ATMs) and transporters associated with antigen processing (TAPs). The soluble proteins include the 2',5'-oligoadenylate activated RNase inhibitor homologues (RLIs), yeast general control non-repressible homologues (GCNs) and structural maintenance of chromosome homologues (SMCs) [6]. By contrast, the non-intrinsic ABC protein (NAP) subfamily cannot be classified in this way because NAPs are a heterogeneous group of soluble or non-intrinsic membrane proteins [6].

A genome-wide analysis enables the classification of the subfamily members of the ABC gene family on the basis of genomic information. This approach provides basic genetic information on the evolutionary characteristics, diversity and relationship of ABC genes and proteins, which serves as a basic resource for

predicting more functions, detecting the relationship between genes and evolutionary diversity of different species. The complete inventories of plant ABC transporters are available for *Arabidopsis* [2], *Oryza sativa* [8], *Vitis vinifera* [9], *Zea mays* [10], *Brassica napus* [11], *Ananas comosus* [12], *Solanum lycopersicum* [13], *Capsicum annuum* [14], *Hevea brasiliensis* [15] and *Lotus japonicas* [16]. The recent sequencing of the whole genome and a large set of transcriptome analyses of *Salvia miltiorrhiza* leads to the analysis of ABC transporters on a genome scale [17–21].

*S. miltiorrhiza* is a commonly used medicinal plant for the treatment of cardiovascular diseases and inflammations because of its high accumulation of biologically active hydrophilic salvianolic acid (SA) and lipophilic diterpenoids (tanshinones) in its roots or rhizomes [22]. *S. miltiorrhiza* is an ideal model medicinal plant for studying secondary metabolic biosynthesis. GGPP is the biosynthetic precursor of tanshinone, which is catalysed by copalyl diphosphate synthase (CPS) to form copalyl diphosphate, and then, a series of cytochrome P450 monooxygenases (CYP450s) catalyses the downstream oxidation reactions. Ferruginol, the catalytic product of CYP76AH1, is an important intermediate product in the biosynthetic pathway of tanshinone [23]. CYP76AH3 and CYP76AK1 are responsible for the conversion of ferruginol into 11,20-dihydroxy ferruginol and 11,20-dihydroxy sugiol en route to tanshinones [24]. SA biosynthesis is derived from 4-coumaroyl-3',4'-dihydroxyphenyllactic acid (4C-DHPL), which is a combination of 3,4-dihydroxyphenyllactic acid (DHPL) and 4-coumaroyl-CoA. These two compounds are coupled by the rosmarinic acid synthase (SmRAS) [25]. The 3-hydroxyl group is introduced by a cytochrome P450-dependent monooxygenase (SmCYP98A14) to form rosmarinic acid [25]. Significant progress has been made in the biosynthetic pathways of these active ingredients in *S. miltiorrhiza*, but the transport and storage mechanisms of these compounds in cells have not yet been elucidated.

Here, we described the first complete analysis of the ABC transporter superfamily from the *S. miltiorrhiza* genome. A total of 114 genes, divided into seven subfamilies, were annotated to encode the ABC transporter proteins in *S. miltiorrhiza*. We characterised all the ABC proteins of *S. miltiorrhiza* and performed a phylogenetic analysis in comparison with the members of the *Arabidopsis* ABC superfamily. On the basis of the co-expression analysis of key enzyme genes involved in the biosynthetic pathway of the active ingredients of *S. miltiorrhiza*, we predicted that three ABCG and one ABCC subfamily ABC transporter genes were involved in the transport of the bioactive metabolites of tanshinone and SA, respectively. In addition, the ABC proteins involved in the transport of plant hormones, secondary metabolites, ions and other substances were predicted on the basis of phylogenetic analysis and gene expression profile characteristics of *S. miltiorrhiza*.

## Results

### Identification of ABC transporters in the *S. miltiorrhiza* genome

A total of 204 homologous ABC transporters were annotated in the *S. miltiorrhiza* genome on the basis of the sequence alignment with all the ABC transporters in the *Arabidopsis* TAIR11 database. The ABC transporters of *S. miltiorrhiza* were verified by confirming the integrity of the conserved domains and

motifs of ABC proteins. Finally, 114 genes encoding ABC transporters were identified in the *S. miltiorrhiza* genome (Table 1). Considering that a typical full-sized ABC protein contained at least 1,200 amino acid residues [2], these ABC transporters ranged in length from 186 to 1,978 amino acid residues in *S. miltiorrhiza* (Table 1), indicating that some predicted ABC proteins with short sequences might be pseudogene or not full-length ABC transporters. Amongst the 114 ABC transporters, 86 were intrinsic membrane proteins with TMDs. Of these intrinsic membrane proteins, 50 were putative full-sized ABC transporters, containing at least two TMD and two NBD domains, which were distributed in ABCB, ABCG and ABCC subfamilies (Table 1). Of the other 36 intrinsic membrane proteins, 31 proteins were half-sized ABC transporters, with one TMD and one NBD domain, primarily distributed in the ABCF, ABCG and ABCI subfamilies (Table 1). The remaining five proteins were non-integrated proteins harbouring two TMD domains and one NBD domain or two NBD domains and one TMD domain, most of which belonged to ABCB and ABCG subfamilies (Table 1). In addition, the remaining 28 genes were identified as non-intrinsic proteins, which encoded proteins lacking TMD. Eighteen of these non-intrinsic proteins were covered in five subfamilies (ABCB, ABCD, ABCE, ABCF and ABCG), and 10 proteins encoded a group of non-intrinsic ABC proteins (NAPs) and were divided into the ABCI subfamily (Table 1).

Fifteen motifs of candidate ABC transporters in *S. miltiorrhiza* were predicted and identified by the MEME Web server to characterise the diversity of ABC proteins. The results showed that the conserved motifs amongst the ABC proteins were similar. For example, the motifs of ABC signatures, Walker A and Walker B were present in these proteins (Additional file 1: Figure S1). Moreover, the motifs of the ABC proteins belonging to the same subfamily were distributed in the same position (Additional file 1: Figure S1). The integrity of the full-sized transporter could be checked by analysing the arrangement of these three motifs in the ABC transporters. The ABC proteins with high similarity had the same motif and gene structure, whereas ABC proteins containing different motifs indicated the diversity of gene functions. Moreover, the subcellular localisation of these ABC transporters has been predicted, together with the gene names (Additional file 2: Table S1), indicating that most of these transporters were located in the plasma membrane.

### **Phylogenetic analysis and subfamily classification**

Phylogenetic analysis was used to confirm the subfamily classification of *S. miltiorrhiza* ABC transporters. The 114 ABC transporters were divided into eight subfamilies, including 3 ABCA, 31 ABCB, 14 ABCC, 2 ABCD, 1 ABCE, 7 ABCF, 46 ABCG and 10 ABCI transporter genes (Fig. 1). The distribution of the ABC subfamily of *S. miltiorrhiza* was similar to that of other plants, and the number of genes in the ABCG subfamily was significantly higher than that of other subfamily. A phylogenetic tree was constructed using the ABC transporter of *S. miltiorrhiza* and the ABC proteins identified in other plants to infer the function of the transporter of *S. miltiorrhiza*. Such functional ABC proteins identified in other plants were listed in Additional file 3: Table S2.

### **Subfamily analysis of ABC transporters in *S.miltiorrhiza***

#### **ABCA subfamily**

The plant ABCA subfamily included one full-sized ABC one homolog (AOH) and several half-sized ABC two homologs (ATH). In *Arabidopsis*, AtABCA1, also known as AtAOH1, was the only full-sized ABCA transporter and was the largest ABC protein encoding 1,882 amino acid residues with domains arranged in a forward direction (TMD1-NBD1-TMD2-NBD2) [2, 8]. AtAOH1 was probably involved in pollen germination and seed lipid accumulation and mobilisation [6]. ATHs were half-sized transporters with domains ranging in forward direction (TMD1-NBD1). These transporters were only found in plants and prokaryotes [26, 27]. The expression of AtATH14 and AtATH15 was down-regulated when the seedlings were grown in hydroponic culture with long-term NaCl treatment [28].

Three genes (SMil\_00000810, SMil\_00004803, SMil\_00004804) were annotated to be ABCAs in the *S. miltiorrhiza* genome (Fig. 2). SMil\_00000810 was a full-sized ABCA transporter with high sequence homology to AtABCA1, namely, SmABCA1 (Table 1). SmABCA1 was also a large ABC transporter of *S. miltiorrhiza*, encoded by 1,978 amino acid residues. AtABCA1 might play a role in lipid accumulation and transport during seed maturation and germination in *Arabidopsis*, respectively [6]. Compared with other tissues, SMil\_00000810 was highly expressed in the roots of *S. miltiorrhiza* (Table 1), implying that SmABCA1 might have a similar function to AtABCA1 in *Arabidopsis*. On the contrary, SMil\_00004803 and SMil\_00004804 were half-sized transporters and might be ATH homologues in the *S. miltiorrhiza* genome.

### ABCB subfamily

The ABCB subfamily, the second largest subfamily, consisted of full-sized members, known as MDR or P-glycoprotein (PGP), and half-sized transporters TAP and ATM [7]. The domains of ABCB transporters were arranged in a forward direction: TMD1-NBD1-TMD2-NBD2. A full-sized MDR encoded approximately 1,200 amino acid residues [2]. Most MDRs from animals were plasma membrane efflux pumps, which were capable of transporting amphiphilic cations and cationic phospholipids [29]. AtMDR1 (AtPGP1) was the first cloned and identified ABC transporter with multiple herbicide tolerances in plants [30]. MDR played an important role in bidirectional auxin transport [31], stomatal regulation [32] and metal tolerance in *Arabidopsis* [33], most of which were located in the plasma membrane of plants [34]. ATM was involved in the biogenesis of Fe-S clusters in the mitochondria [35].

Thirty-one genes were assigned to the ABCB subfamily in *S. miltiorrhiza*, 17 of which were full-sized transporters (Table 1; Fig. 3). SMil\_00017134 (SmABCB10), SMil\_00002175 (SmABCB11) and SMil\_00005832 (SmABCB13), encoding full-sized transporters, had sequence homology with *Arabidopsis* AtABCB1 [36] and AtABCB19 [37] (Fig. 3) and OsABCB14 [38] and tomato SlABCB4 [39], all of which were involved in auxin transport. The expression profiles of these three transporter genes had no tissue specificity in *S. miltiorrhiza* (Table 1). SMil\_00020066 (SmABCB30) was highly expressed in the roots of *S. miltiorrhiza*, particularly in the periderm (Table 1). The tissue-specific expression of SmABCB30 was similar to that of the berberine transporter CjABCB2 in *Coptis chinensis* [40], indicating that SmABCB30 might be involved in the transport of secondary metabolites of *S. miltiorrhiza*. We also found that SMil\_00018186 (SmABCB29), SmABCB30 and SMil\_00015238 (SmABCB31) showed sequence homology with AtABCB4/AtPGP4 and AtABC21 (Fig. 3), whereas the latter two transporters were

responsible for the auxin transport in *Arabidopsis* [41, 42]. The full-sized transporters of SMil\_00016160 (*SmABCB14*) were highly expressed in the flowers, whereas SMil\_00001053 (*SmABCB28*) and SMil\_00015518 (*SmABCB18*), likely to *SmABCB10*, were actively expressed in the roots (Table 1). SMil\_00009650 was clustered nearly to AtABCB15, which was implicated in auxin transport of *Arabidopsis* [43]. We hypothesised that these SmABCB genes might participate in auxin transport of *S. miltiorrhiza*. The half-sized transporter SMil\_00021941 (*SmABCB9*) was the only ATM homologue in *S. miltiorrhiza*, which was particularly similar to the ATM of AtABCB23, AtABCB24 and AtABCB25 in *Arabidopsis* (Fig. 3). These three transporters in *Arabidopsis* were involved in the biogenesis of Fe/S clusters [35], and their expression was up-regulated after methyl jasmonate (MeJA) treatment, which was similar to the MeJA-induced expression profile of *SmABCB9*. The half-sized transporter SMil\_00007974 (*SmABCB4*) was highly expressed in all organs (Table 1). It clustered closer to AtTAP1 (Fig. 3), which was involved in aluminium sequestration [33]. We hypothesised that SmABCB9 and SmABCB4 might be related to the metal tolerance of *S. miltiorrhiza*.

### ABCC family

ABCC, also known as MRP, was encoded by at least 1,500 amino acid residues and comprised only the full-sized ABC transporter in *Arabidopsis* [6]. Compared with MDR, MRP harboured the ABCC-specific additional hydrophobic N-terminal transmembrane domain (TMD0) [44]. The domains of the ABCCs were arranged in a forward direction (TMD0-TMD1-NBD1-TMD2-NBD2) [6]. Most MRPs in plants were located in the vacuole membrane, and few have been reported to reside on the plasma membrane [10, 45, 46]. MRPs have been identified in *Arabidopsis* and maize [44, 47, 48]. MRP involved heavy metal tolerance [49, 50], stomatal regulation, glutathione S-conjugate transport [51] and phytate storage in plants [46]. In addition, MRP was responsible for the transport of secondary metabolites in several plants. For example, ZmMRP3 transported anthocyanins in corn [52], and CsABCC4 of saffron mediated crocin accumulation in the vacuoles [53].

The transporter genes of the ABCC subfamily were expressed in all organs and tissues of *S. miltiorrhiza* (Table 1). SMil\_00016360 (*SmABCC2*) and SMil\_00016361 (*SmABCC1*) were preferentially expressed in the roots of *S. miltiorrhiza* (Table 1), and they were homologous to AtABCC11, AtABCC12, AtABCC1 and AtABCC2 in *A. thaliana* (Fig. 4). Given that AtABCC1 and AtABCC2 were involved in the transport of folic acid, glutathione S-conjugates and chlorophyll [51], and the function of AtABCC11 and AtABCC12 was unknown, SmABCC1 was predicted to be related with the transport of primary metabolites in *S. miltiorrhiza*. SMil\_00015789 (*SmABCC5*) was constitutively expressed in all organs (Table 1) and clustered with CsABCC4a and AtABCC4 (Fig. 4). Considering that CsABCC4a acted as a crocin transporter located on the stigma vacuole membrane of *Crocus sativus* (saffron) [53], whereas AtABCC4 participated in the transport of folic acid and glutathione S-conjugate to regulate stomatal movement [54], we presumed that SmABCC5 might be similar to its homologous protein in *S. miltiorrhiza*.

SMil\_00002827 (*SmABCC4*) was highly homologous to ZmMRP3 in maize [52] and VvABCC1 in grape [55], and the latter two transporters were related to anthocyanin transport (Fig. 4). Compared with other

organs, the expression of SmABCC4 in the leaves was higher under MeJA induction (Table 1), and this ABC transporter might be involved in the transport of anthocyanin in *S. miltiorrhiza* leaves.

SMil\_00028509 (SmABCC8) was located on another branch near SMil\_00002827 and was highly expressed in the leaves (Table 1), implying that *SmABCC8* might also participate in the transportation of substances in the leaves (Fig. 4). SMil\_00004040 (SmABCC11) was highly expressed in the flowers and roots, and its homologue AtABCC5 in *Arabidopsis* was related to the storage of phytate and loading of InsP6 in the seeds [46], indicating that SmABCC11 might contribute to the accumulation of phytic acid in *S. miltiorrhiza*. SMil\_00020247 (SmABCC13) was highly expressed in the leaves and roots (Table 1) and clustered with AtABCC3 and AtABCC6 (Fig. 4). AtABCC3 and AtABCC6 were related to heavy metal tolerance [56, 57], inferring that SmABCC13 might be involved in the heavy metal tolerance of *S. miltiorrhiza*.

### **ABCD subfamily**

The ABCD subfamily, also known as PMP, was located in the peroxisome membrane. In plants, this subfamily contained full-sized and half-sized transporters. The full-sized transporter AtABCD1 was related to the import of long-chain fatty acyl-CoA into peroxisomes in *Arabidopsis* [58] and transport of 12-oxophytodienoic acid [59] and jasmonic acids [60]. Two ABCD members, SMil\_00013326 (SmABCD1) and SMil\_00009714 (SmABCD2), were found in the *S. miltiorrhiza* genome (Table 1; Fig. 5). *SmABCD1* was constitutively expressed in all organs and was homologous to AtABCD1 (Table 1; Fig. 5). We hypothesized that SmABCD1 had a similar function to AtABCD1 in *S. miltiorrhiza*.

### **ABCE and ABCF subfamilies**

ABCE, also known as RNase L inhibitor (RLI), was a soluble protein with only two conserved NBDs and without any detectable TMD, which was conserved in eukaryotes and archaea. In *Arabidopsis*, AtABCE1 and AtABCE2 (AtRLI2) were involved in RNA interference (RNAi) regulation other than transport [61, 62]. AtRLI2 catalysed the conversion of mRNA and participated in the biogenesis of the ribosome and initiation of translation in *Arabidopsis* [62]. ABCF, also known as a GCN homologue, similar to ABCE, was a soluble protein containing only two fused NBDs. Yeast GCN20, as the translational regulator of GCN4, elongated the ribosome during the activation of eIF2a kinase GCN2 [63].

Only SMil\_00000396 (SmABCE1) was assigned to the ABCE subfamily of the *S. miltiorrhiza* genome, and it was constitutively expressed in all organs (Table 1; Fig. 5). Based on the functions of their homologues AtABCE1 and AtABCE2 in *Arabidopsis*, SmABCE1 might play roles in the regulation of gene silencing. The ABCF subfamily of *S. miltiorrhiza* contained seven members, four of which (SMil\_00025510, SMil\_00013582, SMil\_00023741 and SMil\_00004895) were highly expressed in all organs (Table 1; Fig. 5). Amongst the members, SMil\_00013582 (*SmABCF6*) was significantly expressed in high abundance in the leaves and was down-regulated after treatment with MeJA (Table 1). Considering that the homologues of SmABCF6 in yeast and humans were involved in the regulation of gene expression [63], SmABCF6 might negatively regulate the expression of leaf tissue-specific genes under MeJA-induced conditions.

## ABCG subfamily

The ABCG subfamily was the largest subfamily in plants, which was represented by full-sized PDR and half-sized WBC transporters. The NBD-TMD domains of this subfamily were arranged in opposite directions. Most of the characterised ABCGs were located in the plasma membrane [64, 65]. To date, SpTUR2 was an early identified transporter protein of the ABCG subfamily, which involved in the transport of sclareol and resistance of herbicides [66]. In addition, the transporters of the ABCG subfamily were related to the transport of paraquat, thereby changing the tolerance of plants to herbicides [67]. ABCG transporters were widely involved in the transport of various compounds in plants [68, 69]. Lr34 was involved in the resistance of wheat to various fungal pathogens [70], and CrTPT2 was responsible for the transport of vinblastine in *Catharanthus roseus* [71]. CsPDR8 and CsPDR12 were related to the hormone response of cucumber [72]. StPDR2 conferred resistance to the biotic and abiotic stresses in tomato [73], and PhPDR2 was identified as a petuniasterone transporter in leaves and trichomes of *Petunia hybrida* [74]. Four PDR genes have been identified in tobacco, amongst which, NpABC1 and NtPDR1 were involved in the secretion of antifungal terpenoids [75, 76]. NbABCG1/2 was involved in the export of antimicrobial diterpenes and capsidiol for defence against *Phytophthora infestans* [77], and *NtPDR3* was induced to express iron deficiency in the culture medium [78].

WBC was involved in the transport of epidermal wax (AtABCG11) [79], plant hormones (ABA, IBA, cytokinin) [69], pathogen resistance (AtPDR8) [80] and kanamycin resistance (AtWBC19) in *Arabidopsis* [81]. WBC was also responsible for the synthesis of pollen walls (AtABCG1 and AtABCG16) [82], lignin biosynthesis [83] and exine formation on the pollen surface (AtABCG26) [84]. GhWBC1 was considered as an important factor for cotton fibre development and cotton elongation [85].

ABCG was also the largest subfamily of ABC transporters in *S. miltiorrhiza*, including 20 PDRs and 26 WBCs (Table 1; Fig. 6). Four genes (SMil\_00010949, SMil\_00020022, SMil\_00027268 and SMil\_00004712) had tissue-specific expression profiles in this subfamily, all of which were highly expressed in the roots of *S. miltiorrhiza* (Table 1). Notably, SMil\_00027268 (*SmABCG4*) was the most highly expressed gene in the periderm of *S. miltiorrhiza* roots (Table 1). Given that tanshinone was synthesized and accumulated in large amounts in the roots of *S. miltiorrhiza*, particularly in the periderm tissues [24], these four transporters might be related to the transport of tanshinone in *S. miltiorrhiza*. Phylogenetic analysis showed that *SmABCG4* and SMil\_00010949 (*SmABCG40*) were clustered relatively near to the ginsenoside transporter PgPDR3 [86] and the antifungal terpenoid transporter NpABC1 and NtPDR1 [75, 76] (Fig. 6). SMil\_00020022 (*SmABCG46*) and SMil\_00004712 (*SmABCG44*) were closely related to AtABCG39/AtPDR11 [67] and AtABCG34 [68] in *Arabidopsis*, which participated in the stress response of *Arabidopsis*. MeJA induced the expression of *SmABCG46* and *SmABCG44* at different levels, which was identical to the MeJA induction of *AtABCG34* in *Arabidopsis* (Table 1). Another full-sized transporter, SMil\_00016963 (*SmABCG45*), possessing the same gene structure and abundance as *SmABCG46*, was highly expressed in the roots of *S. miltiorrhiza* (Additional file 1: Figure S1; Table 1). These five genes of the *SmABCG* subfamily might be involved in terpenoid transport in *S. miltiorrhiza*, which might mediate the stress responses of this medicinal plant. SMil\_00023314 (*SmABCG35*) was only

expressed in the flowers, although it has the same gene structure as *SmABCG46*, suggesting that this gene might be involved in the transport of substances in the flowers of *S. miltiorrhiza* (Table 1; Additional file 1: Figure S1).

SMil\_00004104 (*SmABCG32*) was a full-sized transporter and highly expressed in the leaves. Its homologous protein CrTPT2 was responsible for the transport of catharanthine [71], suggesting that *SmABCG32* might be involved in the transport of secondary metabolites in the leaves of *S. miltiorrhiza*. In addition, six half-sized WBCs that were expressed in various organs, including SMil\_00027466 (*SmABCG12*), showed high expression levels in the flowers, and its homologue AtABCG25 participated in the export of abscisic acid [65], indicating that SmABCG12 might be involved in the transport of plant hormones in the flowers of *S. miltiorrhiza*. SMil\_00015148 (*SmABCG19*) was highly expressed in the flowers and was homologous to AtABCG11 [79] and GhWBC1 [85], suggesting that SmABCG19 likely played roles in the transport substances that were related to the growth and development of *S. miltiorrhiza*. SMil\_00000399 (*SmABCG27*) and SMil\_00005271 (*SmABCG28*) showed the same expression patterns, both of which were half-sized proteins and expressed in all organs other than the leaves (Table 1). Their homologue AtABCG14 mediated the root-to-shoot translocation of *trans*-Zeatin in *Arabidopsis* [87]. Thus, *SmABCG27* and *SmABCG28* likely involved in the hormone transport of *S. miltiorrhiza*. SMil\_00010332 (*SmABCG15*) was highly expressed in the leaves and induced by MeJA (Table 1), indicating that *SmABCG15* might participate in the MeJA signal transduction pathway.

### ABCI subfamily

No ABCH subfamily was found in plants; thus, an additional subfamily (ABCI) containing prokaryotic-type ABC proteins was used instead of ABCH. ABCI was designated as non-intrinsic ABC protein (NAP) with only one NBD. The *Arabidopsis* genome contained 15 ABCIs, whereas the rice genome contained 10 members of this subfamily [2, 8]. The ABCI subfamily of *S. miltiorrhiza* consisted of 10 genes (Fig. 7), all of which contained only one soluble NBD. These ABCI transporters were expressed in all organs of *S. miltiorrhiza* (Table 1). SMil\_00009816 (SmABCI4) might be involved in the biosynthesis of Fe/S clusters in the leaves because its expression profile was similar to the homologous gene *AtABCI6* [88]. SMil\_00003192 (SmABCI5) had a high degree of homology with ABCI13, which involved in the formation of plastid lipids [3]. SMil\_00001364 (SmABCI2) showed high similarity to AtABCI1, which was related to the maturation of cytochrome *c* [89]. The results showed that SmABCI2 and SmABCI5 might have similar functions to their homologous transporters in *Arabidopsis*.

### Gene expression profiling analysis

The gene expression profiles of the 114 putative ABC transporters were detected on the basis of the transcriptome data generated from different organs (leaf, stem, flower, root) and tissues (periderm, phloem, xylem) of *S. miltiorrhiza* (Table 1). The relative expression levels of these genes were analysed by the FPKM values verified by transcriptome sequencing in previous studies [24]. According to the gene expression pattern, 13 genes (*SmABCB4*, *SmABCB7*, *SmABCC1*, *SmABCC5*, *SmABCD1*, *SmABCE1*, *SmABCF3*-*SmABCF6* and *SmABCG46*) were highly expressed in all organs. By contrast, 11 genes showed

low expression levels in all organs, including *SmABCA3*, *SmABCB1*, *SmABCB3*, *SmABCC3*, *SmABCC6*, *SmABCC14*, *SmABCF2*, *SmABCG13*, *SmABCG33* and *SmABCI2*. Furthermore, a total of 46 genes were rarely expressed in all organs, including *SmABCB6*, *SmABCB8*, *SmABCB12*, *SmABCB15-SmABCB17*, *SmABCB20-SmABCB23*, *SmABCB25-SmABCB27*, *SmABCB29*, *SmABCB31*, *SmABCC7*, *SmABCC9*, *SmABCC12*, *SmABCD2*, *SmABCF1*, *SmABCG2*, *SmABCG3*, *SmABCG5-SmABCG7*, *SmABCG9*, *SmABCG10*, *SmABCG14*, *SmABCG16-SmABCG18*, *SmABCG20-SmABCG23*, *SmABCG25*, *SmABCG26*, *SmABCG29-SmABCG31*, *SmABCG34*, *SmABCG36*, *SmABCG38*, *SmABCG39*, *SmABCG41* and *SmABCI7*. The expression of some genes showed tissue- or organ-dependent specificity. For example, we found that 14 genes were highly expressed in the roots and root tissues, including *SmABCA1*, *SmABCB2*, *SmABCB5*, *SmABCB9*, *SmABCB30*, *SmABCC2*, *SmABCC4*, *SmABCC11*, *SmABCC13*, *SmABCG1*, *SmABCG4*, *SmABCG11*, *SmABCG40* and *SmABCI5*. Although nine genes were expressed in the flowers, stems and leaves, they were not expressed in the roots and root tissues, such as *SmABCB14*, *SmABCB24*, *SmABCC10*, *SmABCG12*, *SmABCG15*, *SmABCG19*, *SmABCG32*, *SmABCG45* and *SmABCI6*. The 13 genes were preferably expressed in the flowers, stems, leaves and roots but not in the three tissues of the root, including *SmABCA2*, *SmABCB11*, *SmABCB19*, *SmABCC8*, *SmABCF7*, *SmABCG24*, *SmABCG27*, *SmABCG28*, *SmABCG37*, *SmABCG43*, *SmABCI1*, *SmABCI3* and *SmABCI8*. Moreover, six genes were highly expressed in the root rather than in other tissues, including *SmABCB10*, *SmABCB13*, *SmABCB18*, *SmABCB28*, *SmABCG8* and *SmABCG45*. The different expression profiles of these ABC genes suggested that they might perform different gene functions in *S. miltiorrhiza*.

### **Verification of gene expression of candidate transporters in the transport of tanshinone and SA**

The tissue-specific expression of some transporter genes might be related to their function in specific tissues or organs. By contrast, some genes showed indistinguishable expression profiles in all tissues, suggesting that they might play a role in the transport of basic substances in cells, such as transporter genes that were involved in the transport of primary metabolites. Considering that tanshinone and SA were primarily synthesised and accumulated in the roots of *S. miltiorrhiza* [22, 24], we hypothesised that the highly abundant transporter genes expressed in the roots of *S. miltiorrhiza* might be related to the transportation of tanshinone and SA. Based on the characteristics of gene expression profiles (Table 1), we screened out 18 candidate genes that were highly expressed in the roots for qRT-PCR verification (Fig. 8), including 1 ABCA (*SmABCA1*), 5 ABCBs (*SmABCB10*, *SmABCB13*, *SmABCB18*, *SmABCB28* and *SmABCB30*), 4 ABCCs (*SmABCC1*, *SmABCC2*, *SmABCC11* and *SmABCC13*) and 8 ABCGs (*SmABCG8*, *SmABCG27*, *SmABCG28*, *SmABCG40*, *SmABCG44*, *SmABCG45* and *SmABCG46*). Amongst these candidate ABC genes, we found that the expression patterns of SMil\_00020022 (*SmABCG46*), SMil\_00010949 (*SmABCG40*) and SMil\_00027268 (*SmABCG4*) were nearly identical to that of *CYP76AH1* and *SmCPS1*, which were key enzyme genes involved in the biosynthetic pathway of tanshinone (Fig. 9). Moreover, SMil\_00016361 (*SmABCC1*) was co-expressed with *CYP98A14* and *SmRAS*, which encoded the key enzymes in the biosynthetic pathway of SA in *S. miltiorrhiza* (Fig. 9). The subcellular localisation of these four candidate transporter genes related with tanshinone and SA transport in *S. miltiorrhiza* was predicted to be located in the plasma membrane (Additional file 2: Table S1). Therefore, these four

candidate ABC transporters co-expressed with key enzyme genes in the biosynthesis of tanshinone and SA likely participated in the intracellular transport of these two active compounds in *S. miltiorrhiza*.

In addition, the 3D model of these four candidate ABC transporters was created by Swiss-model using 6vxi.1.A (ABCG2) and 6pza.1.A (ABCC8) as templates, which had a broad substrate specificity, in the Swiss database. The amino acid sequence homology between SMil\_00027268, SMil\_00020022, SMil\_00010949 and SMil\_00016361 with the template protein was 25.68%, 24.79%, 25.98% and 30.46%, respectively (Additional file 4: Figure S2).

## Discussion

In plants, the ABC protein was initially identified as a transporter involved in the final detoxification process [90]. Since this discovery, many reports have shown that the function of this type of transporter extends far beyond detoxification. In recent years, ABC transporters and have become a major focus for research in plants. This is not only due to their overall roles in a variety of processes, such as pathogen reaction, surface lipid deposition, accumulation of phytic acid in seeds, and the transport of plant hormones, but more specifically, due to their essential roles in plant growth and development, response to abiotic stress and the interaction between plants and the environment.

In this study, a total of 114 ABC proteins were identified in the genome of *S. miltiorrhiza*, amongst which, 86 members encoded for ABC transporters with TMDs, including 50 full-sized ABC transporters. The number of the ABC proteins and the full-sized ABC transporters of *S. miltiorrhiza* was similar to that of *Arabidopsis* because the *Arabidopsis* genome contained 120 gene ABC proteins, including 51 full-sized transporters [2, 8]. The total number of genes encoding ABC proteins was nearly identical in the two species, despite of the large differences in genome size (615 Mb *versus* 125 Mb) and gene content (30,478 *versus* 25,498) [8, 17]. The identification of *S. miltiorrhiza* ABC proteins and their comparative analysis with the *Arabidopsis* ABC transporters revealed an obvious conservation of ABC transporters between the two species. A single plant species can synthesize thousands of different molecules, and these molecules can be transported across the plasma membrane of one or more organelles, which probably explains the large size of the ABC transporter gene family in plants [91].

On the basis of the phylogenetic analysis, the *S. miltiorrhiza* ABC proteins were grouped into their respective subfamilies, from ABCA to ABCI, except ABCH. Members of ABCG (46 genes), ABCB (31 genes) and ABCC (14 genes) subfamilies were the most abundant, while ABCA, ABCD and ABCE subfamilies were less abundant (Table 1), which was similar to the subfamily distribution of tomato [13] and grape [9]. The members of most subfamilies, except of the ABCI (NAP) subfamily, grouped more tightly with each other than with the members of other subfamilies (Fig. 1). Similarly, some members of NAPs also were not clustered to a group with high homology in *Arabidopsis* [2].

In *Arabidopsis*, various subfamilies of ABC transporters contain different conserved domains and perform various biological functions. Similar to *Arabidopsis* [2] and grape [9], only one full-sized ABC transporter (SMil\_00000810) has the longest gene sequence in the *S. miltiorrhiza* genome, belonging to

the ABCA subfamily, and homologous to AtABCA1 (Table 1; Fig. 2), which implied the function of SMil\_00000810 maybe similar to AtABCA1. There were two members (SMil\_00004803 and SMil\_00004804) belonging to the ATH homologs in the *S. miltiorrhiza* genome, was the half-size transporter (Table 1; Fig. 2), whereas the Arabidopsis genome contains 11 ATH members [2]. The expression of *AtATH14* (AtABCA10) and *AtATH15* (AtABCA11) in Arabidopsis was regulated in response to salt stress [28]. Phylogenetic analyses showed that SMil\_00004803 clustered closest to AtABCA11 in Arabidopsis, while SMil\_00004804 clustered with the group containing AtABCA10 (Fig. 2), indicating that these two ATH homologues may be involved in the stress response of *S. miltiorrhiza*. In *Arabidopsis*, the AtABCB1, AtABCB4, AtABCB14, AtABCB15, AtABCB19 and AtABCB21 have been reported to be involved in polar auxin transport as their mutants showed reduced auxin transport [41–43, 92, 93]. It contributed us to speculate that the functions of the homologous to these Arabidopsis ABCB in *S. miltiorrhiza* involved in the polar auxin transport (Fig. 3). These *S. miltiorrhiza* ABCB transporters included SMil\_00000837 and SMil\_00005832 homologous to AtABCB1, SMil\_00018186, SMil\_00020066 and SMil\_00015238 homologous to AtABCB4 and AtABCB421, SMil\_00009650 homologous to AtABCB15, SMil\_00017134 and SMil\_00002175 homologous to AtABCB19 (Fig. 3). AtABCC1 and AtABCC2 contributed to the tolerance of Arabidopsis to Cd (II) and Hg (II), while in the absence of AtABCC2, AtABCC1 conferred great tolerance to divalent heavy metals [94]. Overexpression of *AtABCC1* in Arabidopsis enhanced the tolerance and accumulation of Cd(II) [94]. Similarly, AtABCC3 [95] and AtABCC6 [56] might also contribute to improve plant heavy metal tolerance. Otherwise, the vacuolar membrane-localized ABC transporters, such as AtABCC1 [47] and AtABCC4 [54], regulated the concentration of folate in the cytoplasm by transporting excess folic acid to the vacuole, indicating that ABCC transporters, such as AtABCC1, are important for folic acid storage. The knockout mutants of Arabidopsis AtABCC5/MRP5 exhibited a low phytate phenotype [46]. The subsequent transport experiments using vesicles isolated from yeast heterologous expressing *AtABCC5* confirmed that AtABCC5 acts as a phytate transporter [46]. These results indicated that ABCC transporters played important roles in transporting of primary products and improving the heavy metal tolerance in plants. These results provide valuable clues for studying the ABCC genes in *S. miltiorrhiza*, such as SMil\_00016361, SMil\_00019856, SMil\_00015789, SMil\_00004040 and SMil\_00000135 (Fig. 4). Like other plants, ABCG was the largest subfamily in the ABC transporter family of *S. miltiorrhiza* (Table 1; Fig. 6). Several members of ABCG subfamily in Arabidopsis, such as AtABCG11, 12, 13, and 32, were involved in the transport of cuticular lipid precursors for cuticle layer formation from epidermal cells to the surface [96]. Whereas the AtABCG25 [65] and AtABCG40 [64] were high affinity ABA transporters. ABCG14 participated in transport of cytokinin [87]. AtABCG36 regulated the sensitivity of plants to the auxin precursor indole-3-butyric acid [97]. Whereas the homologous of AtABCG36, AtABCG37 participated in the secretion of scopoletin and derivatives by Arabidopsis roots in response to iron deficiency [98]. These results are sufficient to show the diversity of gene functions in the ABCG subfamily. It was worth noting that several members of the ABCG subfamily participated in pathogen defense and/or the crosstalk between plants and microorganisms, with secondary metabolite-dependent. The tanshinone and SA in are also secondary metabolites with diverse pharmacological activities in *S. miltiorrhiza*. Some members of the ABCG subfamily may participate in the transport of these active compounds in this medicinal plant.

Gene expression profiles are complex phenotypic features that can reflect the biological processes of target genes involved in metabolism, tissue and organ development and differentiation, and response to environmental changes in plants. In this study, we analysed a subset of the gene expression profiles in several organs/tissues of *S. miltiorrhiza*. Since the genes in the same biosynthetic pathway are generally coexpressed, we compared the expression patterns of all the 18 candidate ABC transporter genes with the upstream genes encoding SmCPS1, CYP76AH1, RAS and CYP98A14, those were key enzymes involved in tanshinone and SA biosynthesis, respectively (Fig. 9). The coexpression analysis suggested that three ABCG (SMil\_00020022, SMil\_00010949 and SMil\_00027268) and one ABCC (SMil\_00016361) members might be involved in transport of tanshinone and SA in *S. miltiorrhiza*, respectively (Fig. 9). The co-expression of this transporter gene and key enzyme genes in the secondary metabolic pathway indicated that these transporters might be involved in the transport of secondary metabolites. For example, *CsABCC4a* and *CsABCC2* were highly expressed in the stigmas of *C. sativus* and enabled to transport crocin in yeast microsomes and were highly co-expressed with total crocin levels and/or *CsCCD2*, which was the first dedicated enzyme in the crocin biosynthetic pathway [53]. ABCG14 was highly co-expressed with cytokinin biosynthesis and was the major root-to-shoot cytokinin transporter [87]. We anticipate that a functional study in the near future will elucidate the molecular and physiological functions of the lead candidate ABC transporter involved in tanshinone and SA transport in this medicinal plant.

In addition, we found and confirmed the existence of tissue-specific gene expression profiles, which represent the predictive tissue-dependent functions of these ABC transporters in *S. miltiorrhiza* (Table 1; Fig. 8). These results provided not only a valuable information for investigating the functions of the ABC transporter gene in *S. miltiorrhiza* but also a practical procedure for screening candidate genes involved in bioactive secondary metabolite transport in medicinal plants based on genome and transcriptome dataset.

## Conclusion

In this study, we identified and analysed ABC transporters in *S. miltiorrhiza* for the first time and provided the fundamental and detailed information about *S. miltiorrhiza* ABC proteins. The information included all the ABC proteins in *S. miltiorrhiza* with the gene IDs, protein topology, gene expression profiles and phylogenetic trees of subfamily members and orthologues in other plants, showing the reported physiological functions. Based on the previous studies on the functions of ABC genes, the functions of some ABC transporters with domain or expression characteristics were hypothesised in *S. miltiorrhiza*. Combined phylogenetic and co-expression analyses identified three genes (SMil\_00020022, SMil\_00010949, SMil\_00027268) and one gene (SMil\_00016361) to be the lead candidates involved in tanshinone and SA transport, respectively. The transporters identified in the ABCB and ABCC subfamilies might be involved in the transport of secondary metabolites of *S. miltiorrhiza*. In addition, the transporters might be involved in the transport of anthocyanins, auxin and metal resistance have been identified in several ABC subfamilies of *S. miltiorrhiza*. Our study outlined the ABC proteins in the *S. miltiorrhiza* genome and explained their possible transporting pathways for some compounds, laying an important foundation for further research on the metabolic regulation, synthetic biology and utilisation of these

compounds in *S. miltiorrhiza*. Our analysis provides new insight into the diversity and the predicted function of the entire ABC transporters in *S. miltiorrhiza* compared with *Arabidopsis*. These results will provide new insights into the function of ABC transporters in *S. miltiorrhiza*.

## Methods

### Identification of ABC transporter genes in the *S. miltiorrhiza* genome

BLAST was used to align all the proteins in the *S. miltiorrhiza* genome with the ABC proteins in the *Arabidopsis* TAIR11 database ( $E$  value is less than  $1e-5$ ) and identify ABC homologues in *S. miltiorrhiza*. HMMER (<https://www.ebi.ac.uk/Tools/hmmer/>) was used to characterise the topology of ABC proteins containing TMD and NBD domains. The TMD domain in these ABC proteins was identified by PF12698, PF06472 and PF00664, and the NBD domain was identified by PF00005 in Pfam database (<https://pfam.xfam.org/>). A protein with at least one NBD domain was predicted to encode an ABC transporter. The domain analysis also provided evidence for the subfamily classification of the ABC family in *S. miltiorrhiza*. The obtained *S. miltiorrhiza* ABC protein sequences were submitted to the MEME Web server (<http://meme-suite.org/>) to confirm the relationship between the conserved motifs and genes. The subcellular location of *S. miltiorrhiza* ABC proteins was predicted by the WOLF PSORT online tool (<https://wolfsort.hgc.jp/>). A 3D model of the selected protein was created using Swiss-model (<https://swissmodel.expasy.org/interactive/>).

### Phylogenetic analyses

Clustal X (<http://www.clustal.org/>) was used to perform sequence alignment on the deduced amino acid sequence of *S. miltiorrhiza* ABC proteins, and then, MEGA 6 was used to construct a phylogenetic tree with 1,000 bootstrap repeats through the neighbor-joining method. The maximum likelihood method was used to establish phylogenetic trees of *S. miltiorrhiza* ABC transporter subfamilies with the ABC transporters that have been functionally identified in *Arabidopsis* and other plants to predict the function of these transporters in *S. miltiorrhiza*. Phylogenetic trees were embellished using the interactive Tree Of Life Platform (<https://itol.embl.de/>).

### Analysis of gene expression profiles using transcriptome data

*S. miltiorrhiza* (line 99-3) plants were grown in the medicinal plant garden of the Institute of Medicinal Plant Development. The transcriptome of different organs (flower, stem, leaf, root), root tissues (periderm, phloem, xylem) and leaves (with and without MeJA treatment) was sequenced by Illumina Hiseq 2000 technology in our previous studies [23–24]. The expression profiles of 112 putative *S. miltiorrhiza* ABC genes were analysed with these transcriptome data. The candidate genes that were highly expressed in the roots of *S. miltiorrhiza* were selected for quantitative reverse transcription polymerase chain reaction (qRT-PCR) verification.

### qRT-PCR verification of gene expression profiles

The relative expression levels of 18 candidate ABC genes in different organs/tissues of *S. miltiorrhiza* were analysed by qRT-PCR. Total RNA was extracted from the flower, stem, leaf, root and tissues of the periderm, phloem and xylem isolated from the roots of 2 year-old *S. miltiorrhiza*, according to the manufacturer's instructions of the RNAprep Pure Plant Kit (TIANGEN, China), and reversed into cDNA using the Promega GoScript Reverse Transcription System (Promega, Beijing, China). qRT-PCR was performed on an ABI PRISM 7500 real-time PCR system (Applied Biosys) using SYBR Premix Ex Taq™ (Takara, Beijing, China) with the following program: 95 °C for 30 s, 1 cycle; 95 °C for 5 s and 60 °C for 34 s, 40 cycles. The relative gene expression level was calculated using the  $2^{-\Delta\Delta CT}$  method [99] with the *SmActin* gene (Genbank number HM231319.1) as an internal reference. The experiments were performed in three independent biological experiments with three technical replicates. qRT-PCR was performed to determine the relative expression levels of the 18 candidate ABC genes along with *CYP98A14*, *SmRAS1*, *CYP76AH1* and *SmCPS1*, which were used as positive genes to calculate the correlation coefficient of co-expression for these ABC genes with the key enzyme-encoding genes involved in the biosynthesis of tanshinones and SAs. The primers used in qRT-PCR were listed in Additional file 5: Table S3.

## Abbreviations

ABA : Abscisic acid; ABC: ATP-binding cassette; AOH: ABC one homolog; ATH: ABC-two homolog; ATM: ABC transporter of the mitochondrion; 4C-DHPL: 4-coumaroyl-3',4'-dihydroxyphenyllactic acid; CPP: copalyl diphosphate; CPS: copalyl diphosphate synthase; CYP450: cytochrome P450 monooxygenase; DHPL: 3,4-dihydroxyphenyllactic acid; GCN: general control non-repressible; MeJA: methyl jasmonate; MDR: multidrug resistance; MRP: multidrug resistance-related protein; NAP: non-intrinsic ABC protein; NBD: nucleotide binding domain; OPDA: 12-oxophytodienoic acid; PDR: pleiotropic drug resistance; PGP: P-glycoprotein; PMP: peroxisomal membrane protein; RAS: rosmarinic acid synthase; RNAi: RNA interference; RLI: RNase L inhibitor; SA: Salvianolic acid; SMC: structural maintenance of chromosomes; TAP: transporters associated with antigen processing; TMD: transmembrane domain; WBC: white-brown complex homolog.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Consent for publication

Not applicable.

## Availability of data and materials

The datasets supporting the conclusions of this article are included with in the article and its additional files.

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## Authors' contributions

HL conceived and designed the work. LY drafted the manuscript and was responsible for the data analysis, collected the sample and performed RT-qPCR. JZ and HC assisted to collected the sample and manuscript revision. All authors read and approved the final version of the manuscript.

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## Additional Files

**Additional file 1: Figure S1.** Motif analysis of the ABC proteins in *S. miltiorrhiza*.

The corresponding motifs of the ABC proteins were shown in different colours identified by the MEME motif search tool. Similar sequences shared more motifs.

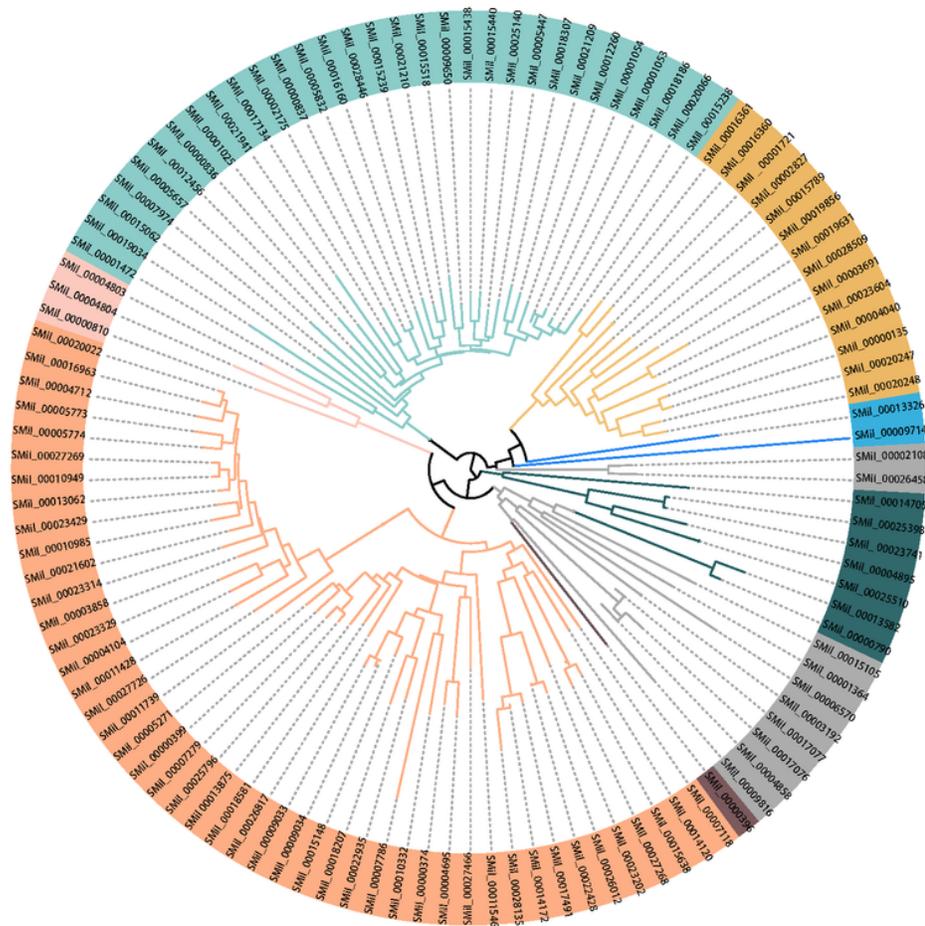
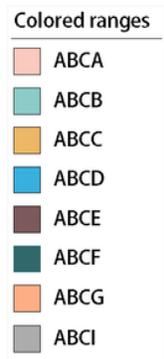
**Additional file 2: Table S1.** Subcellular location prediction by WOLF PSORT of each putative ABC transporter gene in this study.

**Additional file 3: Table S2.** Transporters functionally identified from other plants, including the gene name, corresponding functions and locus ID on NCBI.



performed using MEGA6, with 1000 bootstrap replicates. The Human Genome Organization (HUGO) nomenclature was used. The ABCI subfamily of *S. miltiorrhiza* was not clustered similar to the ABCA-ABCG subfamilies.

Tree scale: 0.1

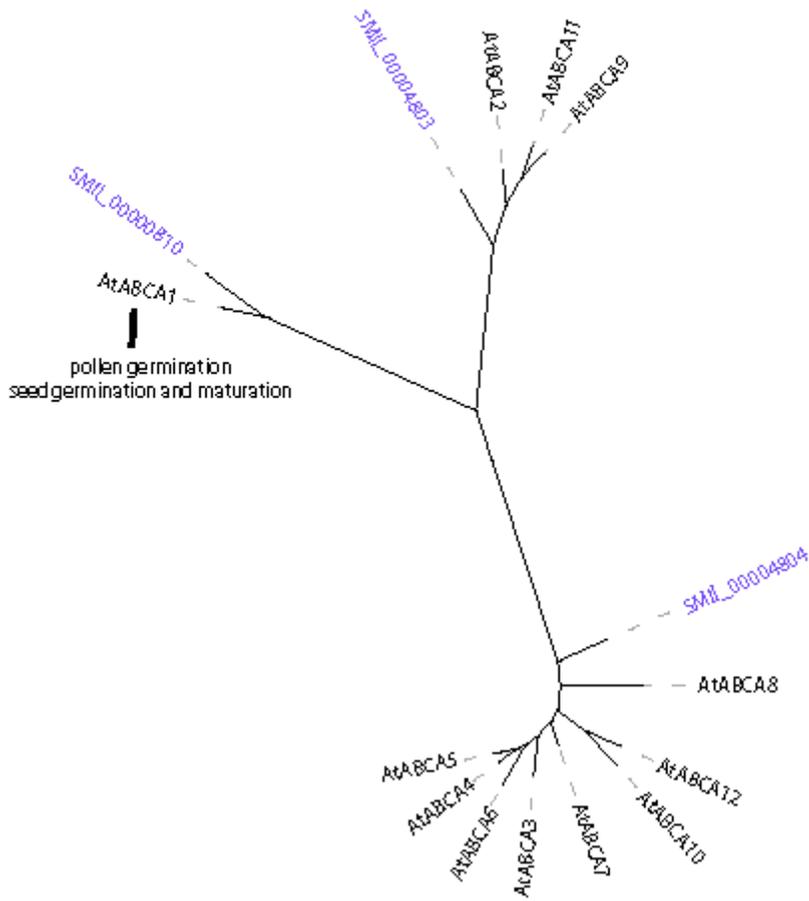


**Figure 1**

Phylogenetic analysis of *S. miltiorrhiza* ABC proteins. Phylogenetic analysis was carried out using the amino acid sequences of the NBD of 112 ABC proteins identified in *S. miltiorrhiza*. The amino acid sequences of the NBD of all the *S. miltiorrhiza* ABC proteins were aligned using the ClustalW program and were subjected to phylogenetic analysis by the distance with neighbor-joining (NJ) method. NJ analysis was performed using MEGA6, with 1000 bootstrap replicates. The Human Genome Organization (HUGO) nomenclature was used. The ABCI subfamily of *S. miltiorrhiza* was not clustered similar to the ABCA-ABCG subfamilies.

# ABCA

Tree scale: 0.1

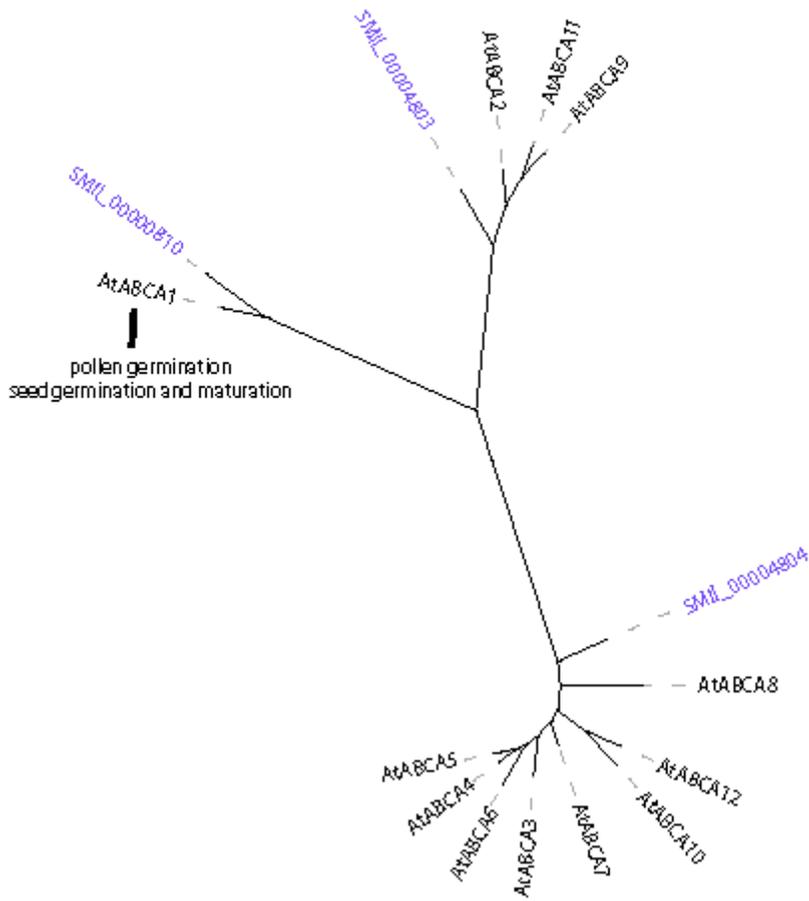


**Figure 2**

Phylogenetic tree of the ABCA subfamily. The ABCAs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCA subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

# ABCA

Tree scale: 0.1

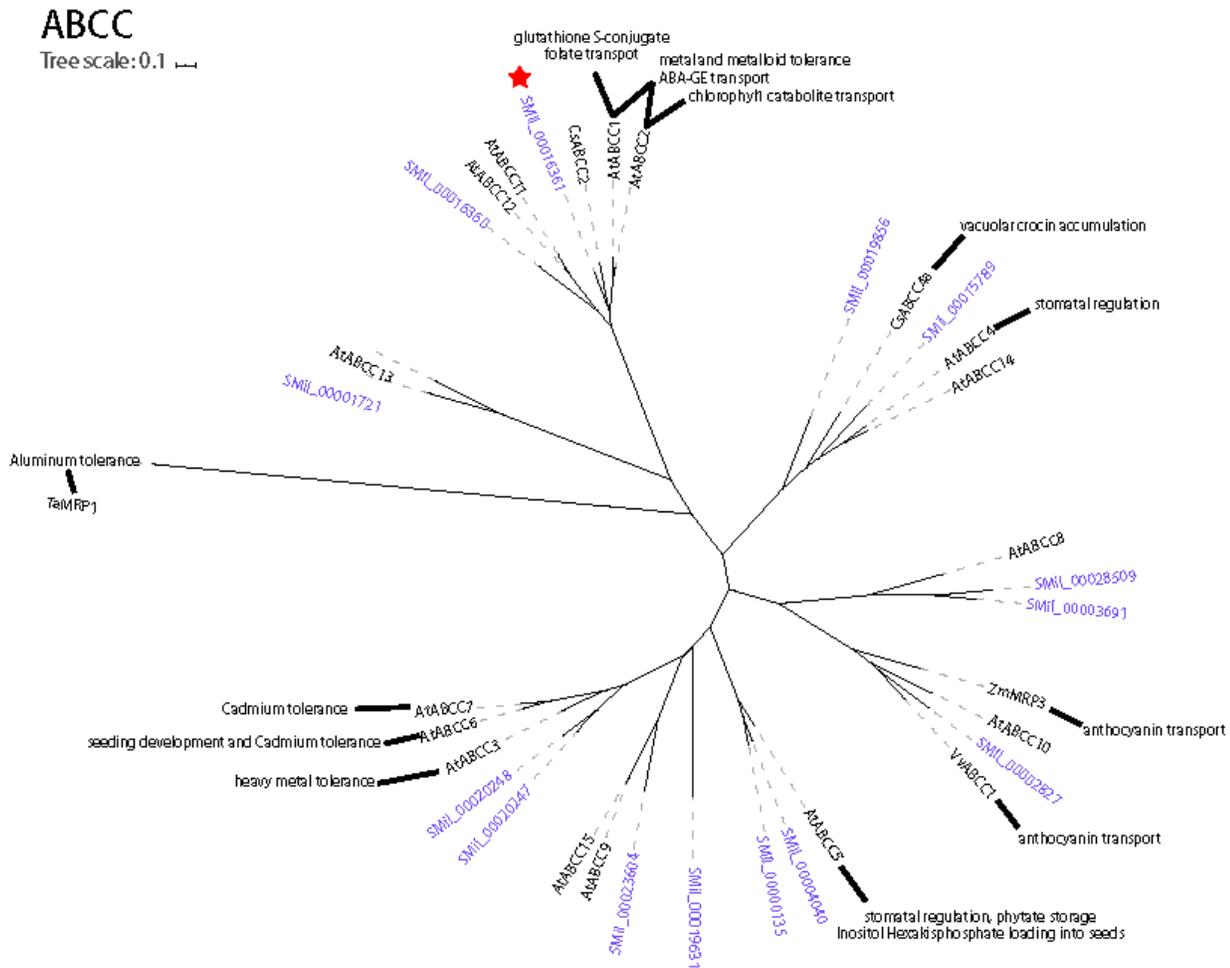


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Phylogenetic tree of the ABCA subfamily. The ABCAs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCA subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

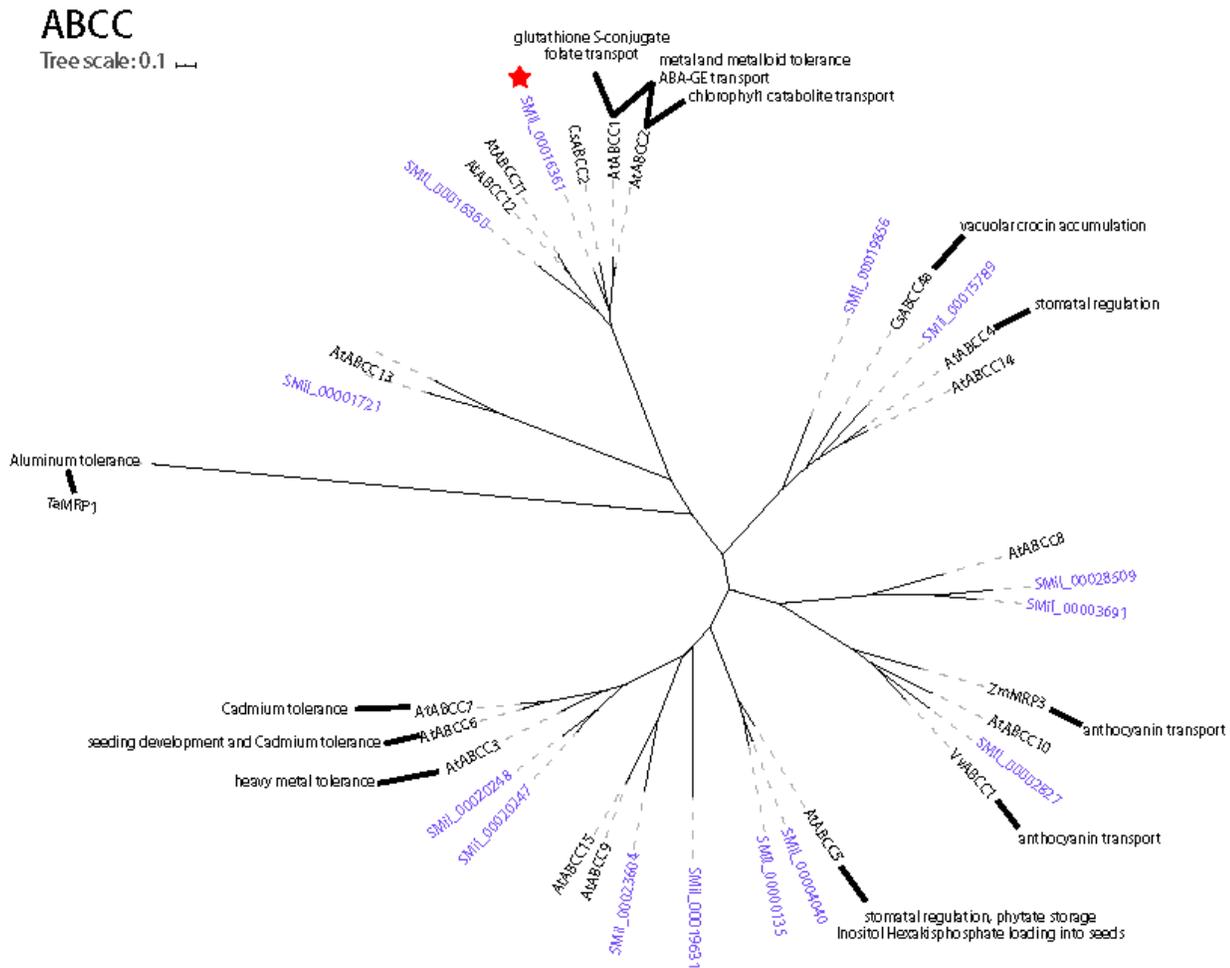






**Figure 4**

Phylogenetic tree of the ABCC subfamily. The ABCCs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCC subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

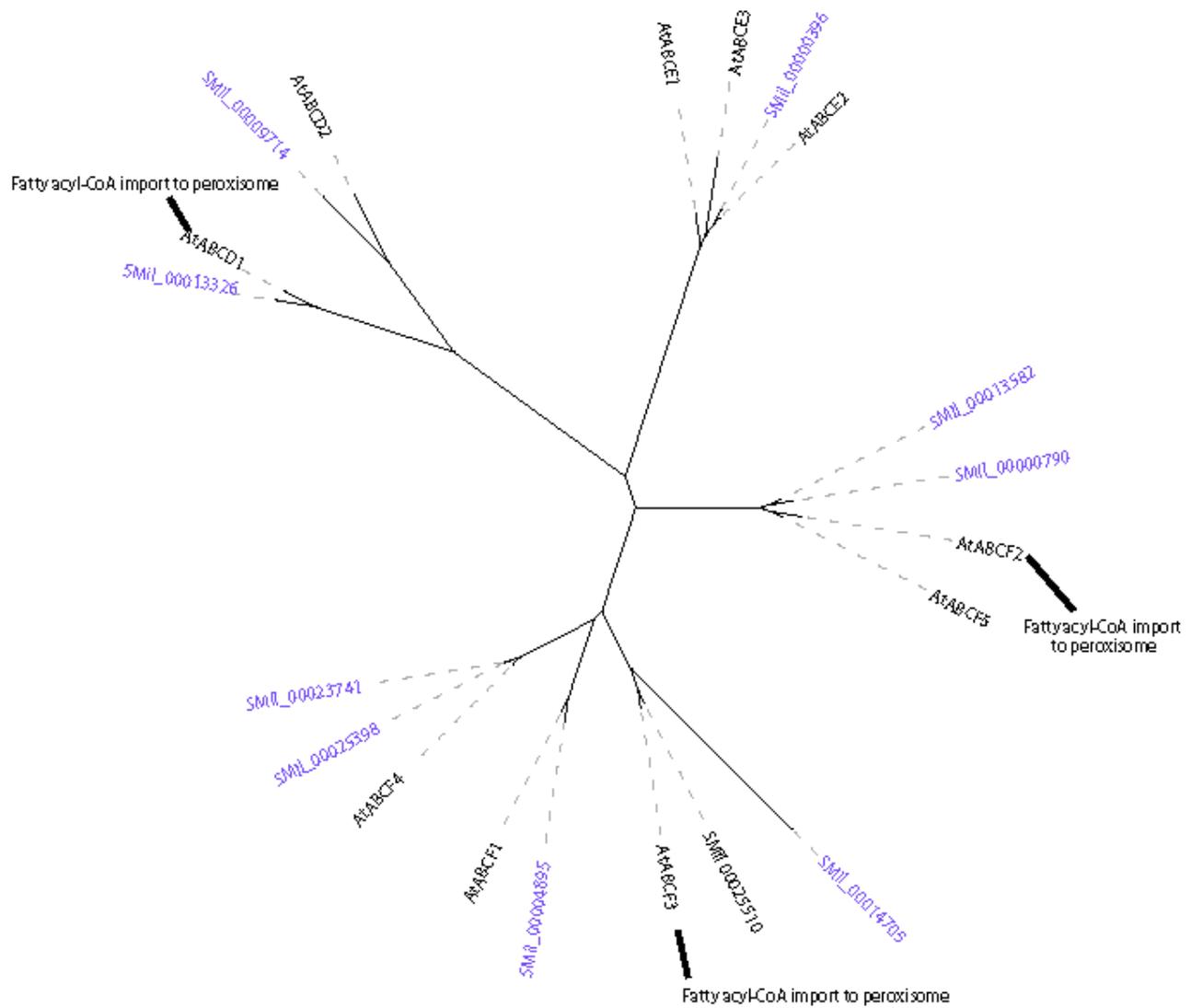


**Figure 4**

Phylogenetic tree of the ABCC subfamily. The ABCCs of *S. multiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. multiorrhiza* ABCC subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

# ABCD/ABCE/ABCF

Tree scale: 0.1

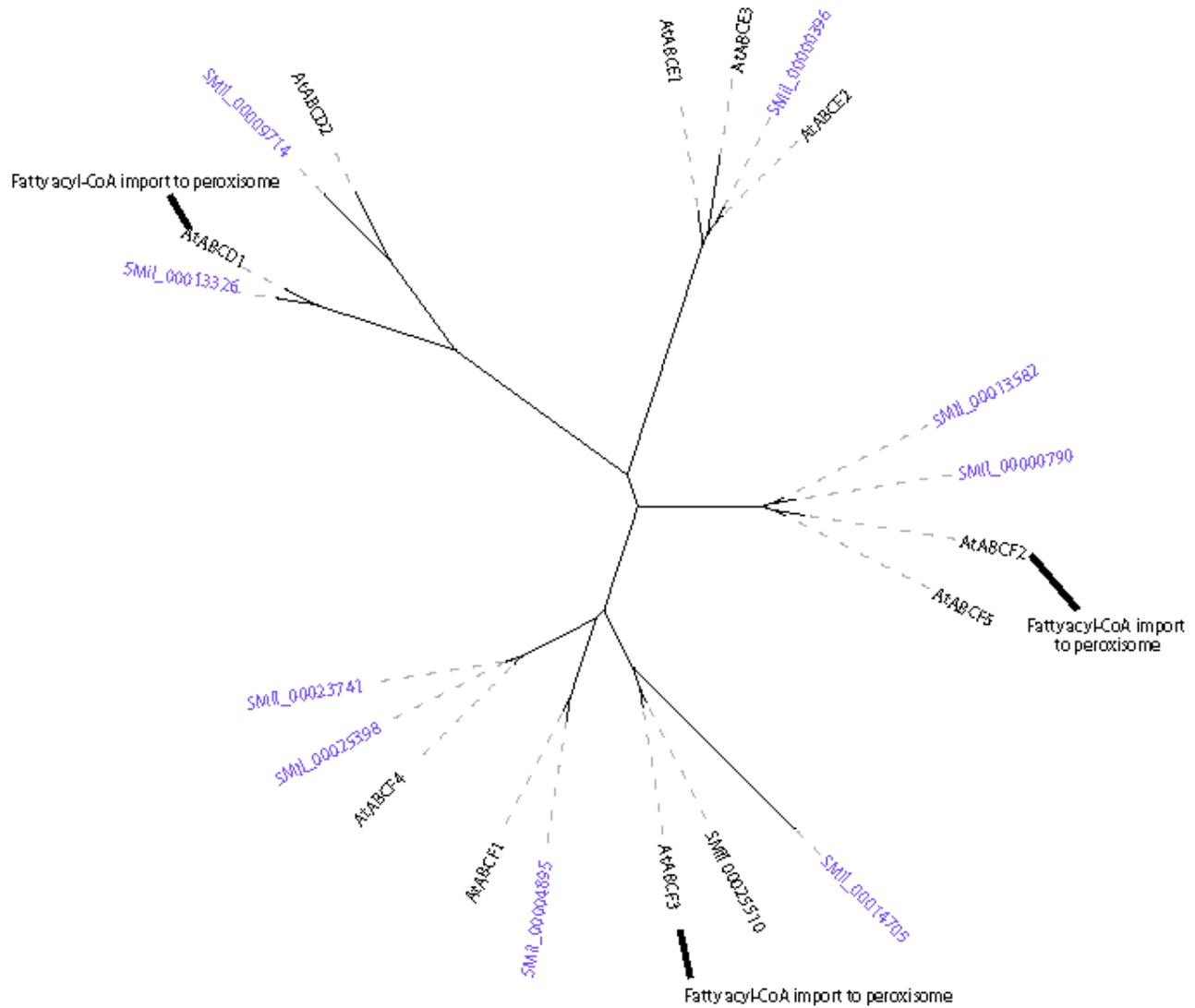


**Figure 5**

Phylogenetic tree of the ABCD, ABCE and ABCF subfamilies. The ABCD, ABCE and ABCFs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCD, ABCE and ABCF subfamilies were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

# ABCD/ABCE/ABCF

Tree scale: 0.1



**Figure 5**

Phylogenetic tree of the ABCD, ABCE and ABCF subfamilies. The ABCD, ABCE and ABCFs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCD, ABCE and ABCF subfamilies were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

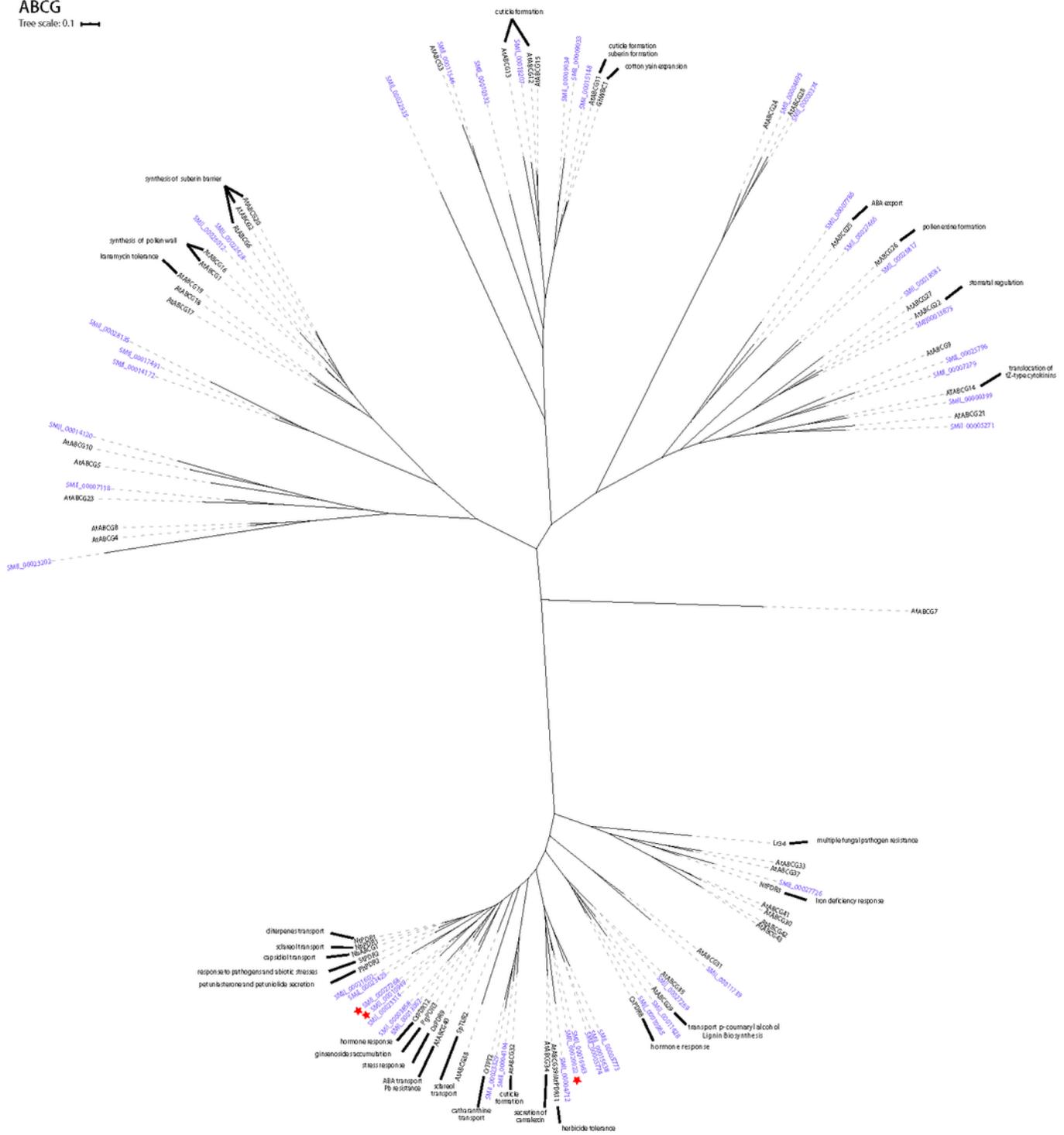
ABCG  
Tree scale: 0.1



**Figure 6**

Phylogenetic tree of the ABCG subfamily. The ABCGs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCG subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

ABCG  
Tree scale: 0.1

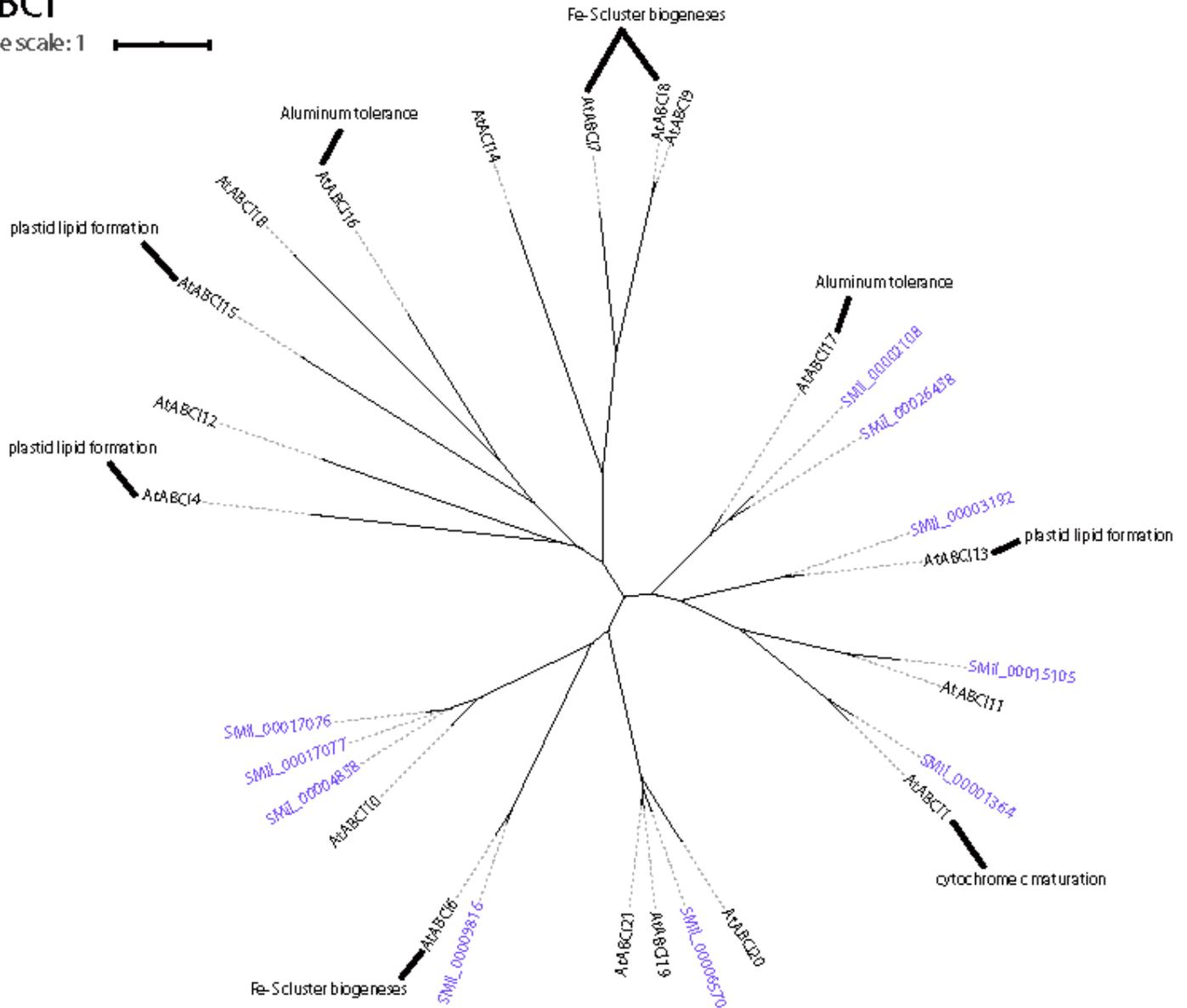


**Figure 6**

Phylogenetic tree of the ABCG subfamily. The ABCGs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCG subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

# ABCI

Tree scale: 1

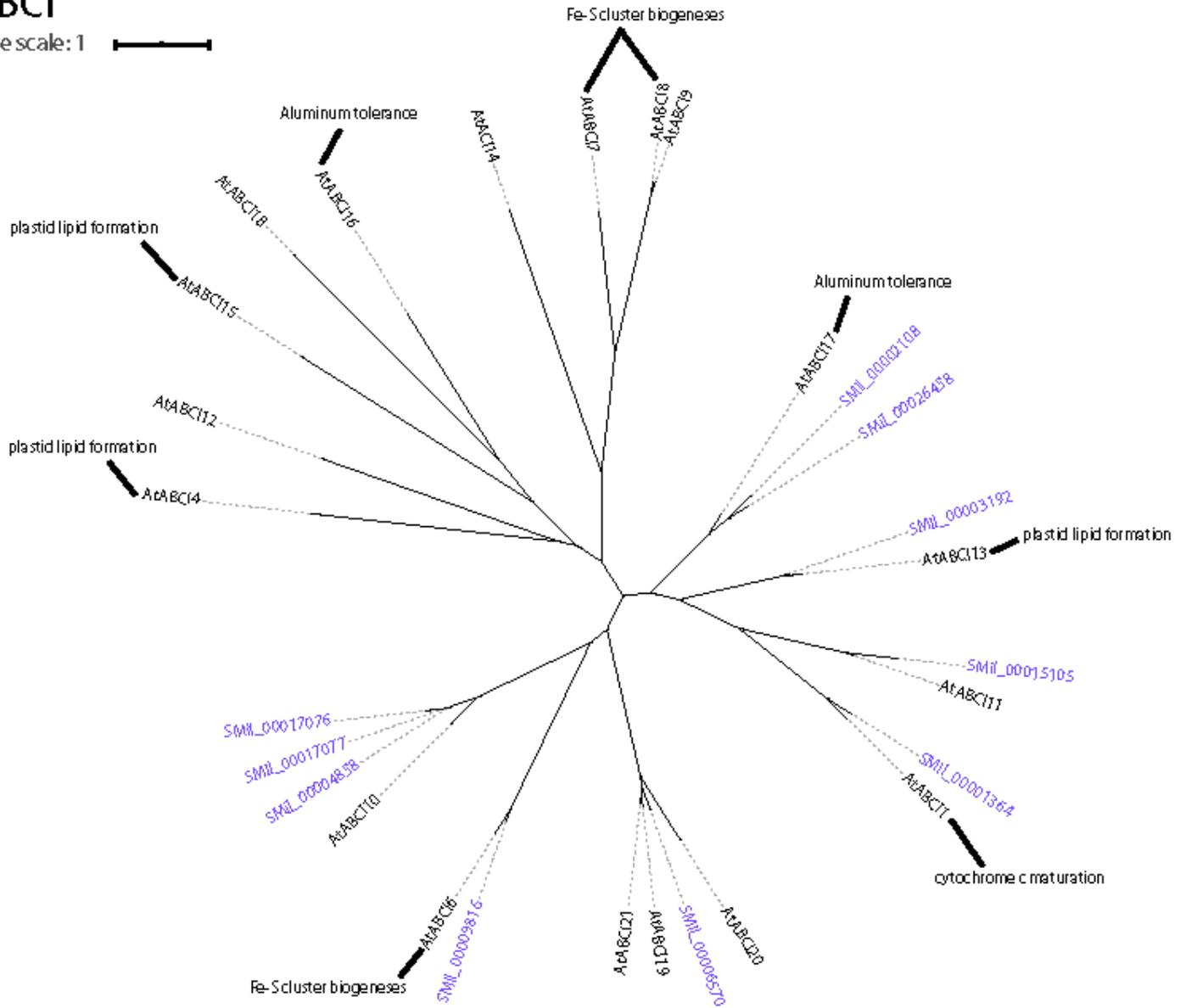


**Figure 7**

Phylogenetic tree of the ABCI subfamily. The ABCIs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCI subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.

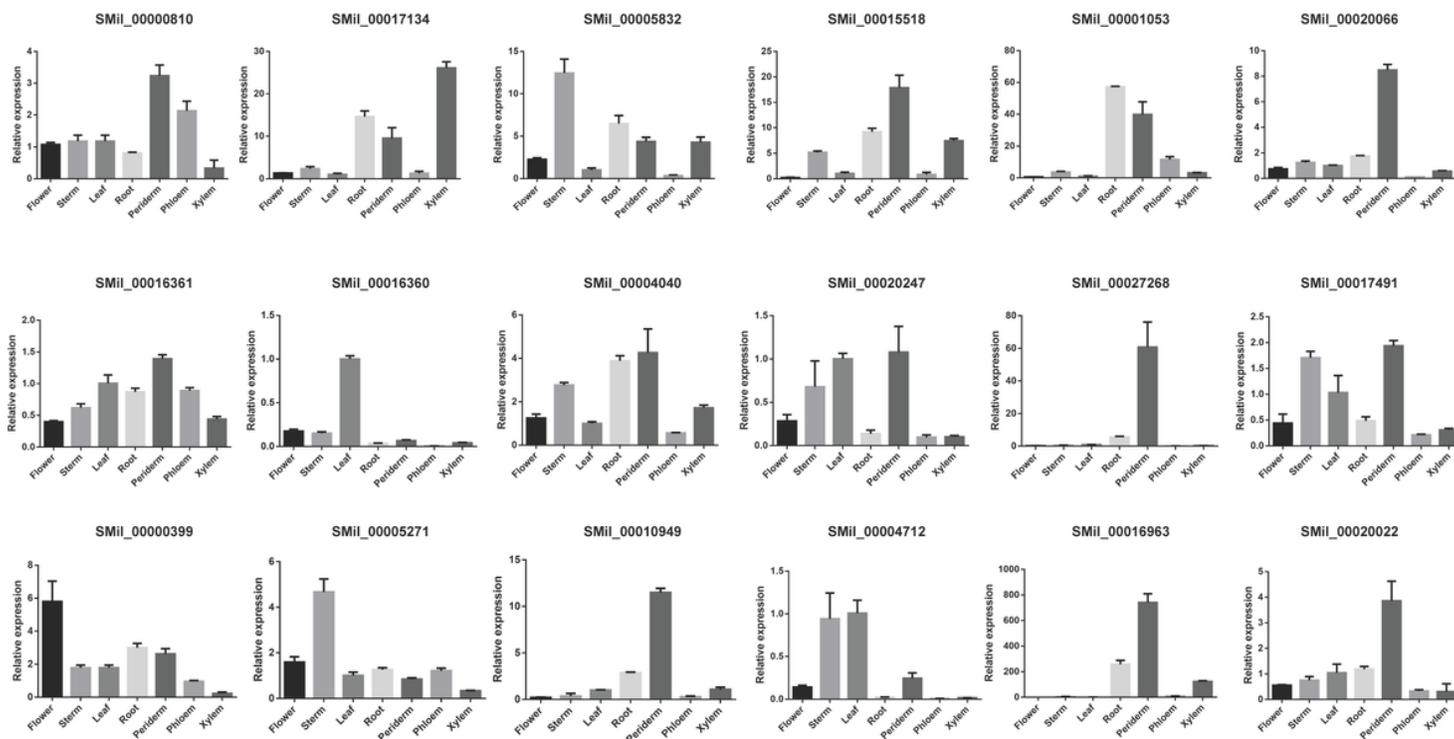
# ABCI

Tree scale: 1



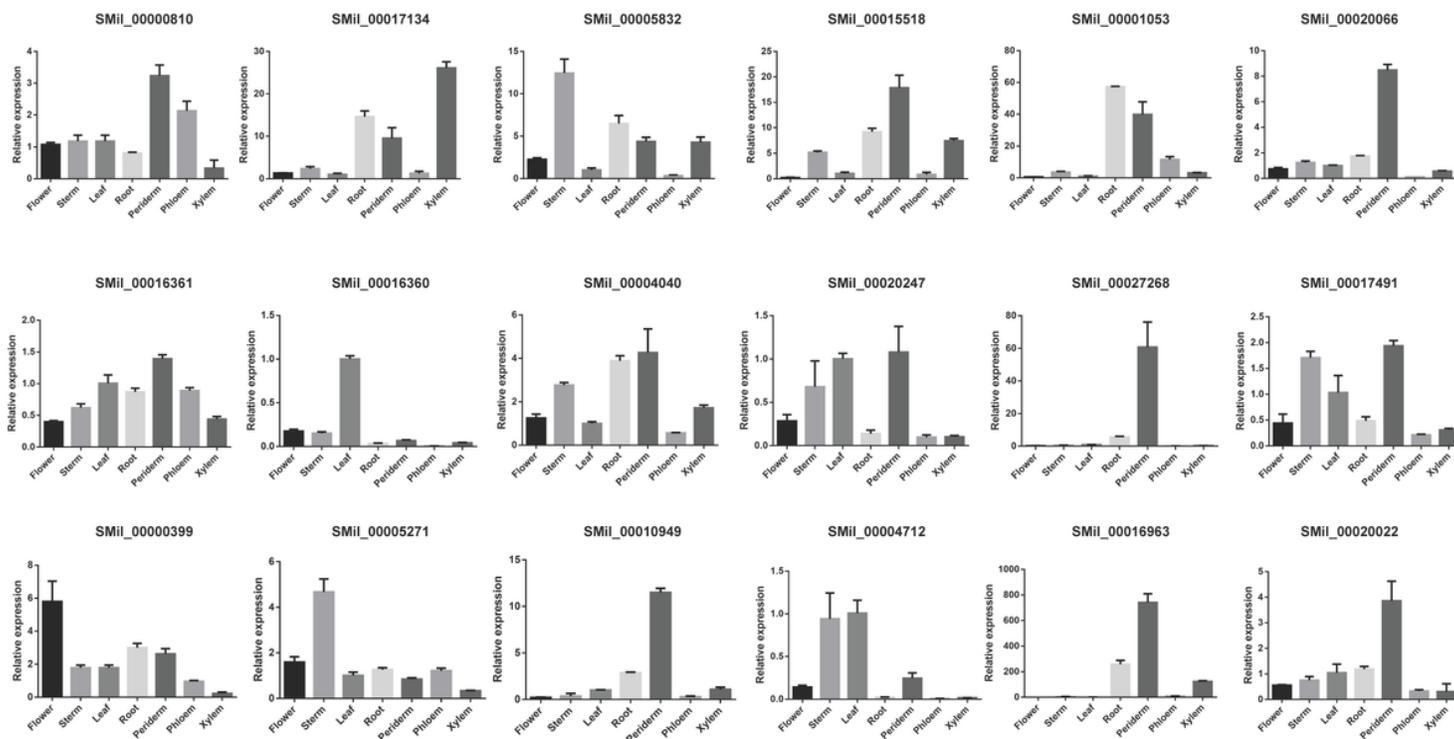
**Figure 7**

Phylogenetic tree of the ABCI subfamily. The ABCIs of *S. miltiorrhiza* and *Arabidopsis* were subjected to phylogenetic analysis. The members of the *S. miltiorrhiza* ABCI subfamily were shown in purple. Physiological functions were indicated. The scale indicated in the figure showed 10% divergence amongst protein sequences.



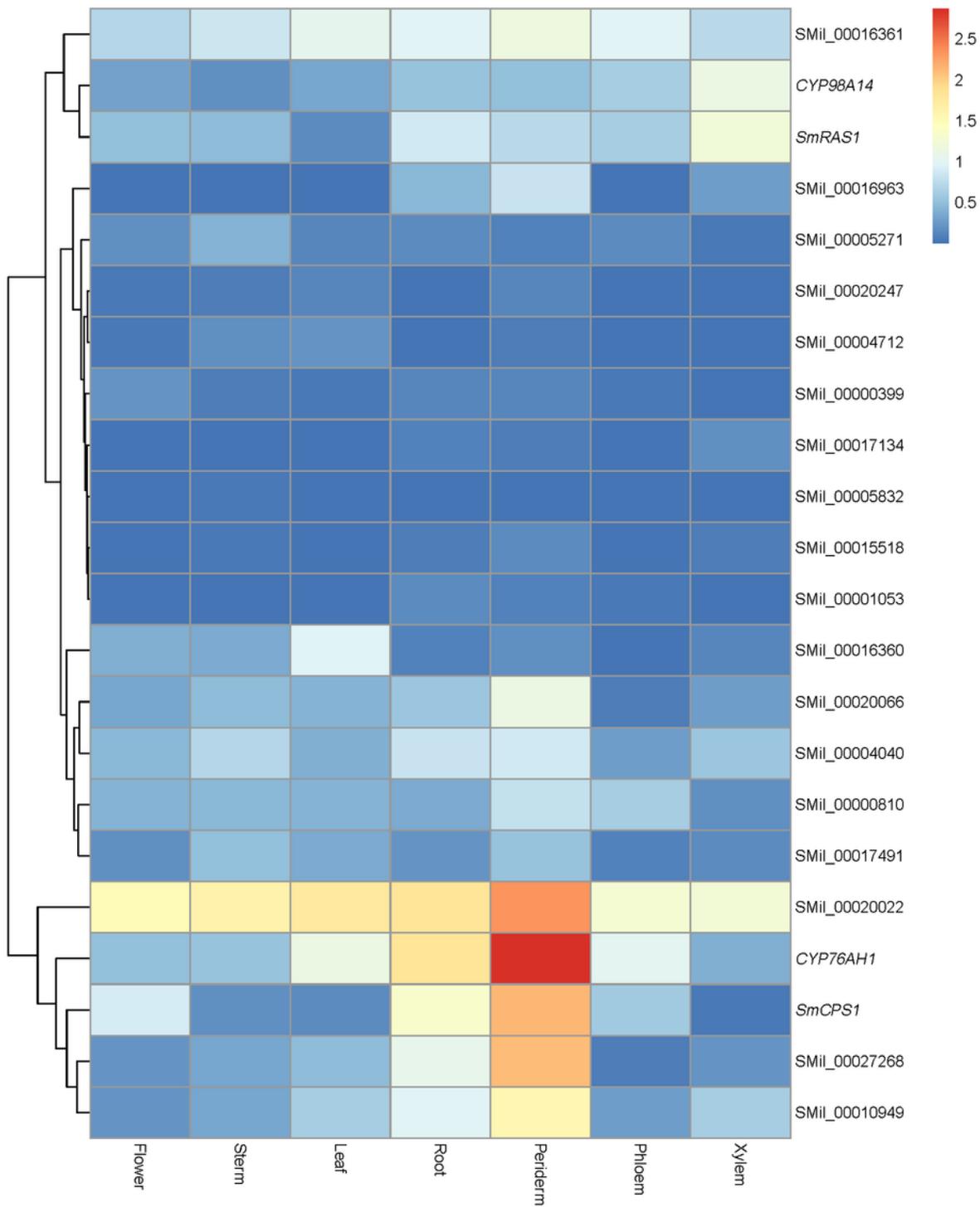
**Figure 8**

qRT-PCR validation of the selected candidate transporter genes involved in bioactive compound transportation in *S. miltiorrhiza*. Expression verification of 18 candidates from *S. miltiorrhiza*. SmActin was used as an internal control. Each gene has three biological replicates and three technical replicates.



**Figure 8**

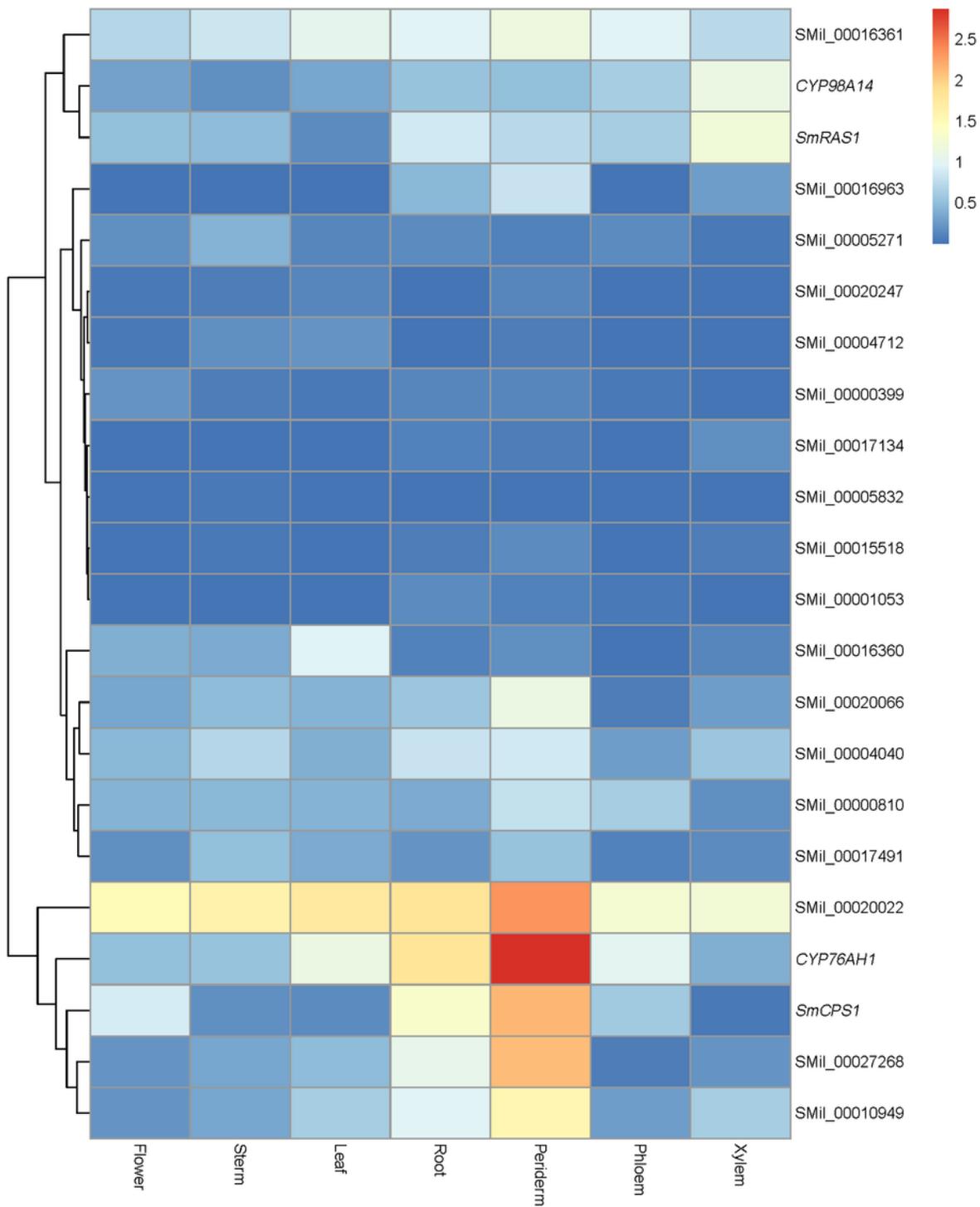
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**Figure 9**

Co-expression profile analysis of candidate transporter genes and key enzyme genes involved in the biosynthetic pathway of tanshinone and salvianolic acid. The heat map showed the relative gene expression profiles of 18 candidate genes detected by qRT-PCR in the organs (root, stem, leaf and flower) and tissues of the root, including the periderm, phloem and xylem. The gene co-expression patterns amongst the candidate genes with the key enzyme-coding genes involved in tanshinone biosynthesis

(CYP76AH1 and SmCPS1) and salvianolic acid biosynthesis (CYP98A14 and SmRAS1) were calculated and clustered by a hierarchical method.



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Co-expression profile analysis of candidate transporter genes and key enzyme genes involved in the biosynthetic pathway of tanshinone and salvianolic acid. The heat map showed the relative gene expression profiles of 18 candidate genes detected by qRT-PCR in the organs (root, stem, leaf and flower)

and tissues of the root, including the periderm, phloem and xylem. The gene co-expression patterns amongst the candidate genes with the key enzyme-coding genes involved in tanshinone biosynthesis (CYP76AH1 and SmCPS1) and salvianolic acid biosynthesis (CYP98A14 and SmRAS1) were calculated and clustered by a hierarchical method.

## Supplementary Files

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