Vegetable Research

ARTICLE

Open Access

https://doi.org/10.48130/vegres-0025-0042

Vegetable Research **2025**, 5: e049

Genome-wide analysis of the ATP-binding cassette B subfamily in tomato and its response to cadmium stress

Pian Yang, Guixiang He, Quanwu Liang, Ge Song, Yuqiong Li, Yan Gao and Jihong Zhang*

Hunan Key Laboratory of Economic Crops Genetic Improvement and Integrated Utilization, College of Life Science, Hunan University of Science and Technology, Xiangtan 411201, China

* Corresponding author, E-mail: jihongzh01@hnust.edu.cn

Abstract

The ATP binding cassette transporter (ABC) superfamilies consist of many membrane proteins and play a very important role in transmembrane transport of substances such as secondary metabolites, phytohormones, and heavy metals. However, it is still unknown what role the B subfamily of the ABC family (ABCB) plays in tomatoes. In the present study, we conducted a genome-wide analysis of the ABCB subfamily in tomato (*Solanum lycopersicum*), which is the largest group in the ABC family with 29 members, followed by the C subfamily. The predicted ABCB genes were distributed across 10 chromosomes, with up to six *SlABCB* genes emerging on chromosome 3 and one *SlABCB* gene detected on chromosome 4. The quantity and structure of exons and introns varied by gene. Analysis of the transcriptome data revealed increased transcript levels of ABCB subfamily genes following cadmium stress. Similarly, these results of quantitative real-time polymerase chain reaction (qRT-PCR) were also confirmed. A yeast experiment showed that the genes *SlABCB8*, *SlABCB15*, *SlABCB22*, and *SlABCB26* can mediate Cd tolerance. Overall, our research offers new insight into the molecular function of ABCB subfamily transporter genes in *S. lycopersicum*, which is highly valuable and important because of the effect of long-term application of *ABCB* transporter genes on regulation under heavy metal stress.

Citation: Yang P, He G, Liang Q, Song G, Li Y, et al. 2025. Genome-wide analysis of the ATP-binding cassette B subfamily in tomato and its response to cadmium stress. *Vegetable Research* 5: e049 https://doi.org/10.48130/vegres-0025-0042

Introduction

Metallic cadmium (Cd) is a by-product of lead, copper, and zinc refining plants. It is recorded that about 20 million hectares of land are polluted by cadmium and other heavy metals in China[1]. ABC-binding cassette (ABC) transporter proteins exist in all organisms, and their family members are mainly divided into three categories: Full molecular transporters, half-molecular transporters, and soluble transporters[2,3]. They release energy by binding and hydrolyzing adenosine triphosphate (ATP) and transport various substrates across cell membranes. The substrates which they transport mainly include metal ions, polypeptides, small proteins, inorganic molecules, alkaloids, and lipids[4,5]. The full molecular transporter consists of two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). However, the half-molecular transporter has merely one TMD and one NBD[2,6,7].

The ABC proteins of eukaryotes can be grouped into eight primary subfamilies (A_H)[6]. Both *Oryza sativa* and *Arabidopsis thaliana* have more than 120 ABC transporter genes within their genomes[8]. In *Arabidopsis*, 21 whole-molecule ABCB subfamily proteins were identified and grouped into three clusters[9]. The primary function of *At*ABCB21 is to regulate auxin distribution and serve as a supplement to the main auxin transporters ABCB1 and 19 in the ABCB subfamily[10]. The loss-of-function mutants of ABCB15, 16, 17, 18, and 22 (ABCB15–22), which are novel auxin-transporting ABCBs, showed strongly reduced the lateral root density[11]. In plants, distribution of auxin is done by auxin transporters, which include PIN, PILS, Aux1/LAX, and some other ABCB members[12].

Furthermore, under heavy metal pollution, plants regulate their growth by altering the resource allocation to various organs to better adapt to adverse conditions and survive[3]. The tap-type transporter *At*ABCB27 (ALS1/TAP2) is localized to the vacuolar membranes and is crucial for root development in environments with severe aluminum pollution[13]. Microscopic analysis with a Cd-sensitive probe revealed that *At*ABCC1 and *At*ABCC2 could improve plants' resistance to Cd by transferring Cd from the cytoplasm to the vacuole[14]. In rice, the TAP transporter *OsABCB27* (*Os*ALS1), which is localized in the vacuolar membrane, is involved in the aluminum stress response[15]. *Os*ABCG36, a Cd efflux transporter, is considered to have functionally redundancy with *Os*ABCG44[16]. In rice, the expression of *Os*ABCG53/*Os*PDR20 is necessary for decreasing the accumulation of cadmium[17]. However, the expression of tomato ABCB transporter genes in response to Cd stress remains unclear.

In this study, we provide comprehensive information on the B subfamily of ABC proteins in tomato under cadmium stress. Analyses of the exon–intron organization, chromosomal distribution, motif analysis, comprehensive phylogenetics, and distribution of *cis*-regulatory elements have been attempted. This study may provide a foundation for further functional analysis of the ABCB transporter's role in resistance to cadmium in *S. lycopersicum*.

Materials and methods

Identification of putative ABC transporter family members in tomato

The characteristic domains of the ABC transporter family were obtained from the Pfam database (http://pfam.xfam.org). Biological sequence analysis with the HMMER software was carried out to construct the hidden Markov model (HMM) and acquire an amino acid database for ABC transporters[18]. The NCBI website and SMART software were used to identify the candidate proteins for AAA domains and ABC2 transmembrane domains and to eliminate the sequences for the missing domains[19].

Phylogenetic analysis

A multiple-step strategy was utilized to construct the phylogenetic tree. The analysis of large protein families is particularly challenging, since they may include several domains and repetitive sequences.

Amino acid and nucleotide sequences of the tomato ABC family were compared with ClustalW. Through the neighbor-joining (NJ) tree methods and maximum likelihood, the rootless phylogenetic tree was built using MEGA 6.0 software, and each had 1,000 bootstrap replicates. The differences between the nucleotides of the *SlABCB* genes were computed with MEGA 6.0[20,21]. The evolutionary tree was further developed via the Evolview website (www.evolgenius.info/evolview). The genome annotation files of *Arabidopsis* (TAIR10.1) and tomato (SL 4.0) were obtained from NCBI. MCScanX was used to investigate the whole-genome collinearity of tomato and *Arabidopsis*[22], and TBtools was utilized to map the whole-genome collinearity.

Identification of conserved motifs and gene structure

With the MEME (Multiple EM for Motif Elicitation) web server, 10 motifs of the SlABCB protein sequences were identified. MEGA X was used to construct their evolutionary relationships[23]. The evolutionary relationships, domains, and motifs of the tomato ABCB gene family were analyzed and integrated using TBtools[24].

Analysis of chromosomal localization and promoter elements

According to the tomato genome annotation files (GFF4.0, general feature format), versions ITAG4.0 and SL3.0 of the genome sequence were downloaded from the Sol Genomics Network (SGN, https://solgenomics.net). The location of the ABCB family members on the tomato chromosome was identified using TBtools software[24]. The ABCB family's physicochemical properties in tomato were predicted with the ExPASy-ProtParam tool.

The promoter sequences of all ABCB genes located in the 2,000 bp sequence of the 5'-upstream region were downloaded from the SGN website. The PlantCARE database was used to predict the promoter elements. TBtools was subsequently used to construct heatmaps and distribution maps[25].

Plant material and cadmium treatments

The seeds surface of tomato (A57 variety) were disinfected for 30 s with 70% ethanol and then with 15% NaClO for 15 min. Before sowing on solid half-strength Murashige and Skoog media, the treated seeds were thoroughly rinsed with deionized water, and vernalizated for 2–4 days at 4 °C. Plants were grown in a greenhouse at a constant temperature (25 + 2 °C) and photoperiod (16/8 h light/dark cycle) for 7 days, and then transferred to half-strength modified Hoagland nutrient solutions[26] supplemented with $CdCl_2$ to a final cadmium concentration of 10 μM. The leaves and roots of plants treated or not treated with cadmium were harvested after two hours of treatment[27]. Fresh tissues were quickly frozen with liquid nitrogen, and stored at −80 °C for RNA extraction.

Total RNA extraction and RNA sequencing

TRIzol RNA extraction reagent (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA from tomato plants grown *in vitro*, which was analyzed using RNA sequencing (RNA-Seq) and quantitative real-time polymerase chain reaction (qRT-PCR)[28]. RNase-free agarose gel electrophoresis was used to verify the RNA's integrity. RNA concentration was determined with an Agilent 2100 Bioanalyzer (Santa Clara, CA). For this, 2 μg of high-quality total RNA with a RIN ≥ 8 for each genotype and treatment were sent to BGI Genomics Co. Ltd. company (Shenzhen) to proceed with the RNA sequencing analysis.

RNA-Seq was carried out on an Illumina HiSeq 2500 platform. The raw reads obtained were filtered, and the remaining reads were mapped to the tomato genome SL3.0 (ITAG4.0). The expression level of each mRNA transcript was calculated as fragments per kilobase of transcript per million mapped reads (FPKM). According to the instructions of manufacturer, the first-strand cDNAs were synthesized by reverse transcription from RNAs treated with DNase I (Fermentas, Canada) with M-MuLV reverse transcriptase (Takara, Dalian, China). qRT-PCR was carried out with SYBR premix ExTaq (Takara) on an ABI7500 system.

qRT-PCR analysis

To validate the RNA-Seq results and identify the key candidates likely to be involved in cadmium tolerance, six upregulated *SlABCB* genes (*SlABCB4*, *SlABCB8*, *SlABCB11*, *SlABCB15*, *SlABCB22*, and *SlABCB26*) were chosen for qRT-PCR analysis. These particular genes were selected because of their strong induction under cadmium stress, their close phylogenetic relationship to functionally characterized ABCB transporters known to mediate heavy metal or auxin transport in other plants, and their promoter regions enriched with stress and hormone-related *cis*-elements, which imply their potential role in broader regulatory networks. Fluorescence quantitative RT-PCR was performed by adopting the $2^{-\Delta\Delta C_T}$ method, and the results are expressed as the relative expression levels of genes[29]. Table 1 lists the qRT-PCR primers. Heat maps were drawn using TBtools software, with different colors representing the expression levels relative to the average expression level. The original data were normalized by calculating the log2 value of the ratio of the measured expression level to the average expression level. Three biological replicates were used to determine the expression profiles, and the data are expressed as the average value and the standard error of the mean. At a significance threshold of $p < 0.05$, Duncan's multiple range tests were used to determine the differences between the means.

Yeast experiments

The primers used for amplifying the coding DNA sequences (CDSs) of the tomato *ABCB* candidate genes are listed in Table 1. The PCR-amplified fragments and the yeast expression vector pYES2 were restricted with of *BamH1* and *EcoR1*. After the double enzyme

Table 1. Primers used in RT-qPCR analysis

Genes	Locus	Forward primers (5'→3')	Reverse primers (5'→3')
ABCB4	*Solyc02g087870.2.1*	CTACTATACAAATCCCGCAA	TGCCAACATCATCCTCCGAA
ABCB8	*Solyc03g114950.2.1*	AACCGTTCTTGTCATAGC	GTCATCATGTGTGCCACT
ABCB11	*Solyc04g010310.2.1*	CGTTCAATCTGCAAATCGGAA	AAACTCACTGTTAGGACGACT
ABCB15	*Solyc06g009290.2.1*	GGCTTTTGATGGGGGCATTG	GATTTTCGCGTAGAGGGCCT
ABCB18	*Solyc06g009290.2.1*	AACAAGCCTGAAATACCTG	GAAGGCAATAAAAGTTACA
ABCB20	*Solyc09g008240.3.1*	TCTTCATACGGTCGATCACC	CGGCCAAACGAACTAGCTTG
ABCB22	*Solyc09g055350.2.1*	GCCAGAATTATAAGCACTCT	CATTCACAACCTTTGCACCA
ABCB26	*Solyc12g098840.3.1*	GCATCTTTACTAGCGACACC	TCTCCAATCATACCTCGGAC
Ubiquitin	*Solyc10g005560.2.1*	CACCAAGCCAAAGAAGATCA	TCAGCATTAGGGCACTCCTT

digestion products were recovered with a gel extraction kit (Tiangen, Beijing, China), they were then subjected to connection via the T4 RNA ligase and then transformed into the corresponding yeast strain, as described by Elble[30]. The positive monoclonal yeast cells in fresh synthetic dextrose (SD) liquid medium were placed and cultured until they reached the logarithmic growth phase, then the yeast cells were collectedby centrifugation. The collected yeast particles were suspended in sterile water and then diluted to an optical density at 600 nm (OD_{600}) of 1, 0.1, 0.01, or 0.001. Subsequently, equal volumes (15 μL) of the suspension were dropped onto medium plates containing different concentrations of heavy metals as indicated, and were incubated at 30 °C for approximately 7 d, with photos taken.

Results

Genome-wide identification of ABC proteins in tomato

In order to clarify the tomato ABC protein families, a BLAST search of SGN genome database was carried out. We searched all the tomato ABC proteins with version ITAG4.0 of the annotation and version SL3.0 of the tomato genome. Ultimately, 154 members putatively encoding ABC gene transporter families were identified (Supplementary Fig. S1), and phylogenetic trees and functional predictions were subsequently generated. The tomato ABC family was grouped into eight subfamilies (A–I), including 9 ABCAs, 29 ABCBs, 26 ABCCs, 2 ABCDs, 2 ABCEs, 6 ABCFs, 70 ABCGs, and 10 ABCIs. Here, we focused on the ABCB subfamily because of its potential role in the Cd stress response.

With an average of 1,018 amino acids, the predicted proteins containing the ABCB gene ranged in quantity from 108 (*SLABCB28*) to 1,401 (*SLABCB9*). The molecular weights ranged from 11.8 to 142.7 kD, with an average of 110.28 kD. An essential physiological indicator of proteins is the isoelectric point (pI), which is based primarily on the percentage of acidic to basic amino acids. ABCBs had isoelectric points ranging from 5.84 to 9.45 (Table 2). The isoelectric points of most of the ABCB proteins (89.66%) were greater than 7, indicating that ABCB proteins might be alkaline proteins.

Analysis of the number of exons, conserved motifs, and chromosomal locations

The conserved domains of the ABCB subfamily proteins in tomato were analyzed with TBtools software. The fifth motif was present in almost all ABCB subfamily proteins except the five genes *SlABCB24*, *SlABCB25*, *SlABCB27*, *SlABCB28*, and *SlABCB29*, suggesting that Motif 5 may be reasonably well conserved (Fig. 1). Each subfamily member showed some common and some unique motifs. In the cluster of *SlABCB3* and *SlABCB28*, *SlABCB28* had only one motif, namely Motif 4. As shown in Fig. 1 and Table 3, 10 distinct motifs were identified, and a schematic overview of the identified motifs is provided. Interestingly, a similar motif composition is shared by the same subgroups, indicating functional similarities among mem bers of the same subgroups (e.g., *SlABCB4* and *SlABCB13*, *SlABCB15* and *SlABCB18*, etc.).

Gene Structure Display Server (GSDS) analysis of the structure of tomato ABCB family members helped to clarify the evolutionary relationships among the proteins. Genes within the same group usually have a similar structure, for example, *SlABCB1* and *SlABCB2*, *SlABCB16*, and *SlABCB17*. All ABCB family members possessed 1 to 20 exons. *SlABCB28* had only 1 exon, whereas *SlABCB20*, *SlABCB21*, and *SlABCB25* had 15, 16, and 20 exons, respectively, which implied significant diversity in their exon–intron structure (Supplementary Fig. S2). This variation in SlABCBs was primarily caused by differences in exon–intron length, which is one of the predominant

Table 2. Characterization of SlABCBs protein in tomato

Gene Name	Gene ID	AA	MW (kD)	pI	Instability index	Aliphatic index	Structural model
SlABCB1	*Solyc02g071340.1*	1,264	137.7	5.84	35.80	103.71	Whole-molecular transporter
SlABCB2	*Solyc02g071350.2*	1,264	137.1	8.09	35.41	103.32	Whole-molecular transporter
SlABCB3	*Solyc02g087410.2*	1,263	137.5	7.97	40.60	99.70	Whole-molecular transporter
SlABCB4	*Solyc02g087870.2*	1,250	136.1	8.08	36.86	99.42	Whole-molecular transporter
SlABCB5	*Solyc03g005860.2*	1,260	136.7	7.20	36.03	105.92	Whole-molecular transporter
SlABCB6	*Solyc03g093650.2*	1,228	134.3	8.35	33.30	102.94	Semi-molecular transporter
SLABCB7	*Solyc04g010310.2*	1,286	142.3	6.33	46.36	101.18	Whole-molecular transporter
SLABCB8	*Solyc06g009280.1*	1,290	139.3	7.80	36.82	101.57	Whole-molecular transporter
SLABCB9	*Solyc06g009290.2*	1,401	138.6	8.18	35.65	100.28	Semi-molecular transporter
SLABCB10	*Solyc06g072960.1*	1,029	113.1	7.01	38.56	104.26	Semi-molecular transporter
SLABCB11	*Solyc07g018130.1*	1,276	140.2	8.73	36.13	101.71	Semi-molecular transporter
SLABCB12	*Solyc07g064120.1*	1,260	138.0	8.88	37.83	93.87	Semi-molecular transporter
SLABCB13	*Solyc08g076720.2*	1,258	138.2	8.75	35.64	103.51	Semi-molecular transporter
SLABCB14	*Solyc09g008240.2*	1,315	140.0	8.63	36.18	95.56	Whole-molecular transporter
SLABCB15	*Solyc11g067310.1*	1,290	141.4	7.05	35.48	103.07	Whole-molecular transporter
SLABCB16	*Solyc12g098840.1*	1,281	138.6	6.38	34.90	101.88	Semi-molecular transporter
SLABCB17	*Solyc12g098870.1*	1,313	142.72	6.76	37.95	100.58	Whole-molecular transporter
SLABCB18	*Solyc11g067300.1*	1,261	138.47	7.50	32.90	102.02	Whole-molecular transporter
SLABCB19	*Solyc05g013890.1*	955	105.16	9.10	43.42	96.12	Whole-molecular transporter
SLABCB20	*Solyc03g026310.2*	664	67.51	8.94	41.69	102.73	Whole-molecular transporter
SLABCB21	*Solyc03g114950.2*	639	68.34	8.45	36.24	104.42	Semi-molecular transporter
SLABCB22	*Solyc03g122050.1*	673	74.69	7.34	34.75	80.97	Semi-molecular transporter
SLABCB23	*Solyc03g122070.1*	667	73.02	9.45	38.67	101.94	Whole-molecular transporter
SLABCB24	*Solyc09g009910.2*	640	71.08	9.14	34.76	113.40	Whole-molecular transporter
SLABCB25	*Solyc09g055350.2*	726	80.37	9.40	28.52	98.52	Semi-molecular transporter
SLABCB26	*Solyc00g304030.1*	1,081	111.53	9.24	44.24	87.73	Whole-molecular transporter
SLABCB27	*Solyc12g049120.1*	349	38.62	9.03	41.41	103.68	Quarter-molecular transporter
SLABCB28	*Solyc12g049130.1*	108	11.8	8.79	35.36	101.12	Quarter-molecular transporter
SLABCB29	*Solyc12g070280.1*	232	25.82	8.63	29.5	113.55	Quarter-molecular transporter

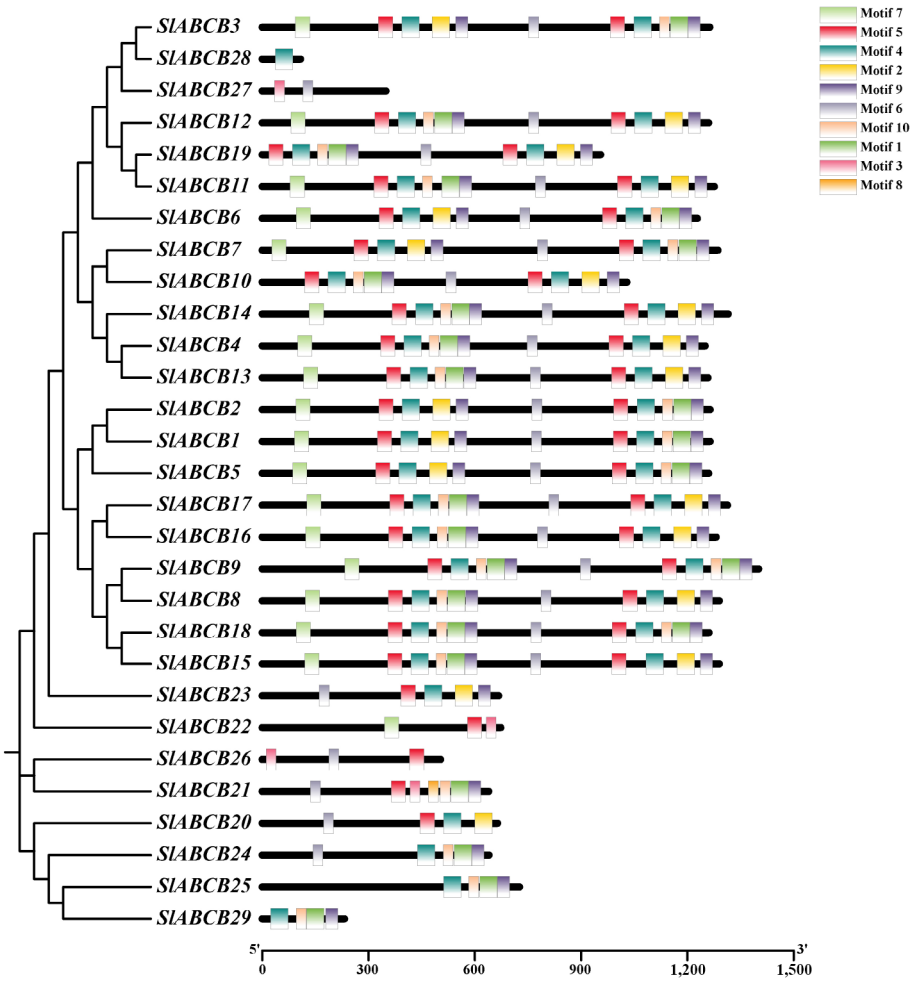

Fig. 1 Phylogenetic tree of ABCB family genes and distribution of conserved protein motifs.

factors affecting gene size. Overall, the conserved motif compositions and similar gene structures of the ABCB members in the same group, together with the results of the phylogenetic analysis, strongly support the reliability of the group classifications.

The chromosomal distribution of the identified ABCB family members revealed that they were distributed throughout 10 chromosomes, with a maximum of six ABCB family members on chromosome 3. There were six members on chromosome 3, and five ABCB genes were located on chromosome 12. In addition, only one ABCB gene member were detected on chromosomes 1, 4, 5, and 8 (Supplementary Fig. S3). The distribution pattern of the ABCB subfamily on individual chromosomes also indicated certain physical regions with a relatively higher accumulation of multiple ABC gene clusters, such as chromosome 3, 11, and 12 at the lower end of the arms. Meanwhile, some ABCB gene clusters were present in the upper chromosome part, such as chromosome 6.

Table 3. The sequences of conserved protein motifs in the ABCB gene family of the tomato genome.

Motif number	Motif sequence
Motif 1	AAKLANAHNFISGLPQGYETQVGERGVQLSGGQKQRIAIARAILKN PKILLLDEATSALDAESERIVQEALDR
Motif 2	EIELKBVYFSYPSRPDVQILNGFSLKIPSGKTVALVGGSGSGKSTVISL JERFYDPQSGZVLJDGVBJKELQLKW
Motif 3	MVGERGTQLSGGQKQRIAIARAILKBPRILLLDEATSALDAESERIVQ EALDRIMINRTTVIVAH
Motif 4	TVALVGESGSGKSTVISLJZRFYDPDSGEIYJDGIDIRKFNLKWLRQQ MGLVSQEPVLFNDTIRENIAYGKEGAAEEE
Motif 5	AGEKLIRRIRSMMFEKVLRMEIGWFDEEENSSGAJGARLSTDAATV RSLVGDRLALJVQBLATAIVALVIAFIASWRLALVMJAVQPL
Motif 6	MVNRTTVVVAHRLSTIKGADVIAVIKBGVIVEKGTHDTLLN
Motif 7	JRQQIGLVSQEPVLFATTIKENIAYGKEDATEEEIKEAIKLANAAKFID KLPZGLDT
Motif 8	KVSLKFVYLAIGVGVASFLZVACWTVTGERQASRIRCLYLKSVLRQD IGFFDTZTNT
Motif 9	GMTLDVIKGBIEFKHVSFAYPTRPDVQILKDLSLTIPAGK
Motif 10	AYEEASQIANEAVGNIRTVASFSAEEKVLELYK

Promoter element analysis

Cis-acting elements within the promoter region act as transcription factors' binding targets, which govern gene expression in a tissue-specific, developmental stage-dependent, or stress-responsive manner. To systematically explore the regulatory-level functional diversity among the ABCB gene family members, 21 *cis*-elements were identified when the ABCB gene promoter sequences were submitted to the Plant CARE database (Fig. 2). Salicylic acid, abscisic acid (ABA), gibberellin, zein, methyl jasmonate (MeJA), and auxin were the main plant hormone-responsive elements in the upstream promoter region.

Anaerobic-, defense stress- and low temperature-induced response elements were among the stress-related *cis*-acting elements evaluated. The greatest proportion of *cis*-related elements related to plant development and growth among all the tomato ABCB family members were light-responsive elements, indicating that light responsive elements might play a significant role in photomorphogenesis.

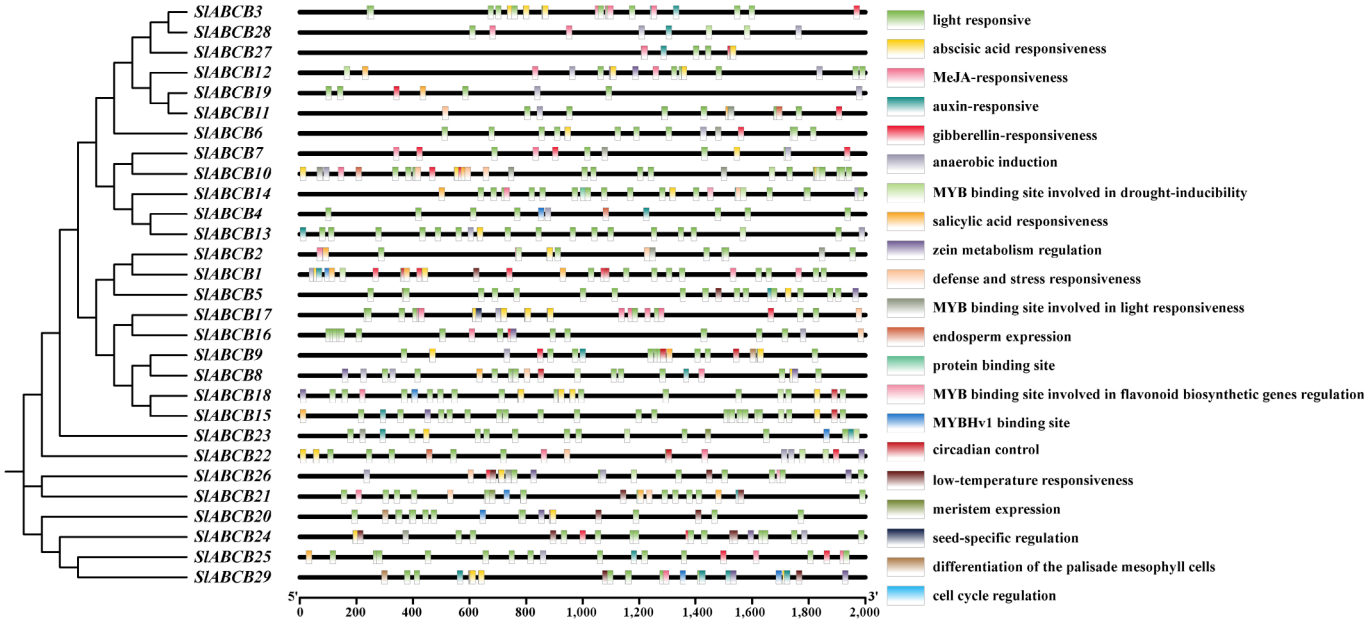

Fig. 2 Location of *cis*-elements in the ABCB genes' promoter sequences.

Gene replication and collinearity analysis of the *SlABCB* genes

Investigating gene replication activities in the tomato genome allowed the investigators to better understand the amplification and evolutionary mechanism of the *SlABCB* gene family. TBtools software was used to investigate the collinearity of the tomato genome. The results revealed that the tomato *ABCB* family included a duplicated gene pair (*SlABCB1* and *SlABCB5*) (Fig. 3). This suggests that there may be functional similarities between *SlABCB1* and *SlABCB5*.

Figure 4 displays the collinear relationships of the duplicated gene pairs in the *ABCB* gene families in the genomes of tomato (*S. lycopersicum*), rice (*Oryza sativa*), eggplant (*Solanum melongena*), and *Arabidopsis thaliana*. Tomato and eggplant (from dicotyledonous Solanaceae) had the most collinear *ABCB* gene pairs, followed by tomato and *Arabidopsis* (from dicotyledonous plants), whereas tomato and rice (from monocotyledonous plants) had the fewest collinear ABCB gene pairs, in accordance with plant evolution laws. Ten pairs of *ABCB* genes were identified between tomato and *Arabidopsis thaliana* (Fig. 4a). *SlABCB13*, *SlABCB19*, *SlABCB21*, and *SlABCB24* were the only pairs of the ABCB gene subfamily found in rice and tomatoes (Fig. 4b). Moreover, 16 pairs of ABCB genes were found to exist between tomato and eggplant (Fig. 4c). These revealed that *ABCB* genes between tomato and eggplant have relatively higher homology and highly conserved sequences.

Phylogenetic relationships of the tomato *ABCB* gene family

A phylogenetic tree was constructed using the *Arabidopsis* ABCB family members to predict the functions of the 29 *SlABCB* genes in tomato and establish their homologies (Fig. 5). Tomato *ABCB* family members can be grouped into seven subfamilies (1–7), which include five, seven, nine, one, one, four, and two *ABCB* gene family members, respectively. There were two groups with the fewest members, Clust4 and Clust5, each including only one protein from the tomato ABCB family (*SlABCB22* and *SlABCB23*). These results suggest that *SlABCB22* and *SlABCB23* may have specific functions in tomatoes (Fig. 5). *SlABCB22* and *SlABCB23* form their own unique clade each, which hints that they may have evolved separate, specialized jobs compared with the other family members. This idea is supported by our functional data for *SlABCB22*, which was strongly induced by cadmium and proved to be very effective at helping yeast survive cadmium stress. It might be a specialized transporter for this metal. The role of *SlABCB23* is less clear, as it did not respond strongly to cadmium. Its unique evolutionary branch could mean it transports something else entirely, perhaps a specific compound that is not related to metal stress.

Transcriptomic analysis of *ABCB* genes across all organs and all stages

The transcriptome data of *ABCB* genes were retrieved and examined to confirm their functions in the tissues and organs involved in reproduction. *SlABCB9*, *SlABCB14*, and *SlABCB21* exhibited relatively high expression levels in *S. lycopersicum* roots, followed by *SlABCB4*, *SlABCB7*, and *SlABCB25*.

SlABCB14, *SlABCB20*, and *SlABCB21* had higher mRNA expression levels than the other genes at all stages. It is worth noting that *SlABCB21* displayed the highest expression in flower buds, followed by *SlABCB14* and *SlABCB20*. *SlABCB20* reached a maximum in the fruit breaker and fruit breaker groups, followed by *SlABCB25* (Supplementary Fig. S4).

RNA-Seq data and qRT-PCR validation

Seven-day-old *S. lycopersicum* plants were exposed to 10 μM Cd^{2+} for 2 hours to measure the expression of *SlABCB* genes under cadmium stress. The RNA-Seq data were processed to determine the expression levels of the *SlABCB* genes (Fig. 6). The results indicated that under cadmium stress, *SlABCB8*, *SlABCB11*, *SlABCB15*, *SlABCB17*, *SlABCB22*, *SlABCB25*, *SlABCB26*, and *SlABCB27* were upregulated.

Six candidate genes that were upregulated in response to Cd stress were selected for qRT-PCR detection to confirm the accuracy of the *ABCB* subfamily of genes in the RNA-Seq data. All six genes (*ABCB4*, *ABCB8*, *ABCB11*, *ABCB15*, *ABCB22*, and *ABCB26*) were induced by Cd exposure (Fig. 7). The trend of variations in the expression of the six ABC transporters, as observed by qRT-PCR, was mostly consistent with transcriptome data. These genes might be related to the heavy metal detoxification mechanisms of tomatoes.

Several *SlABCB* genes mediate yeast's tolerance of Cd

Family members of *SlABCBs* with Cd tolerance were induced and screened via expression in yeast. The metal sensitivity assay was conducted using the wild-type yeast strain Y252 and the yeast

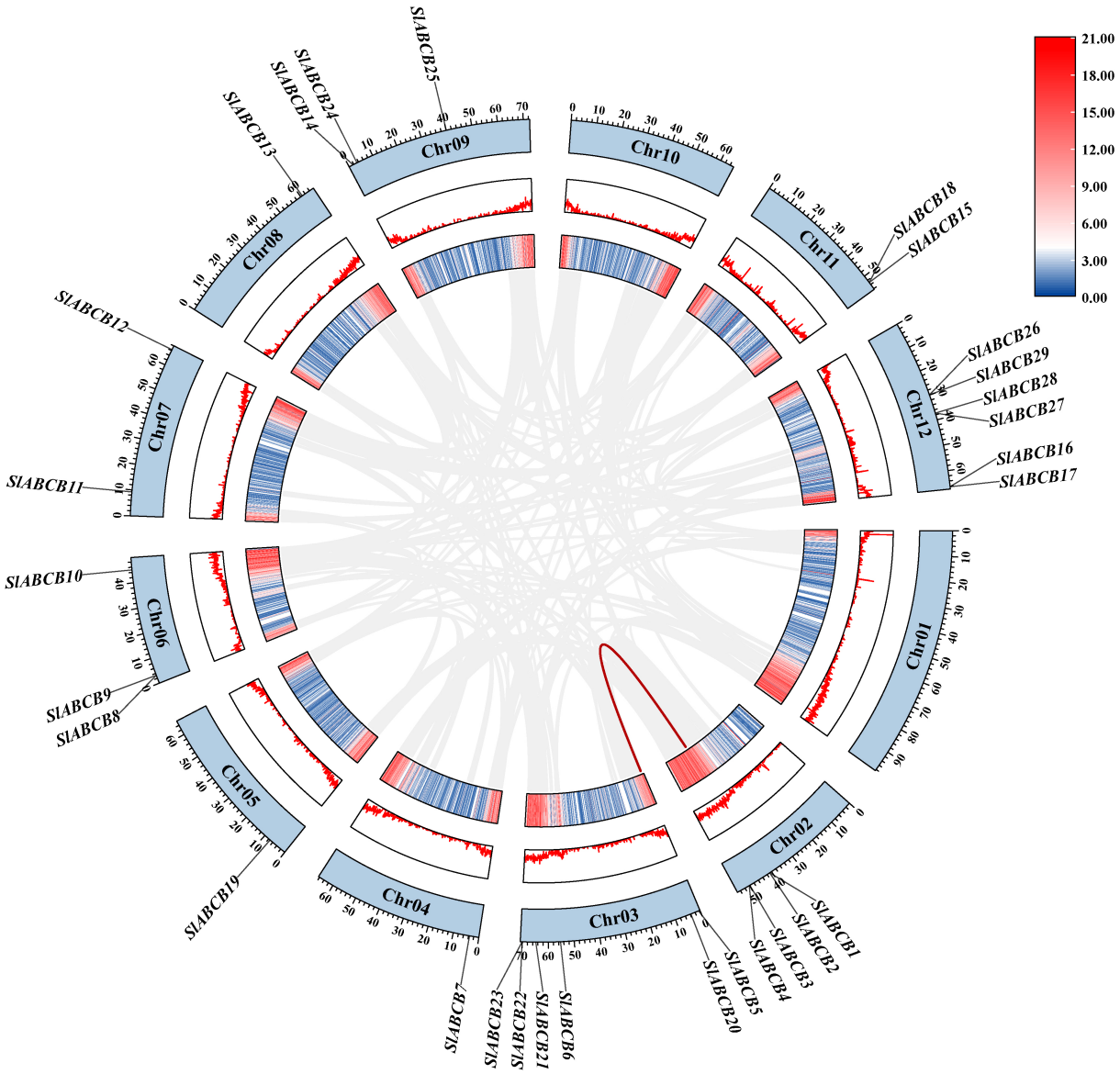

Fig. 3 Collinearity analysis of *ABCB* genes in tomato species from chromosome 1 to 12.

Cd-sensitive mutant strain Δ*yap1*. The research results demonstrated that the Δ*yap1* mutant strains carrying the pYES2-*SlABCB* (*SlABCB8* and *SlABCB22*) plasmids expressed under the control of the GAL1 promoter displayed stronger tolerance to 30 μmol·L^{-1} $CdCl_2$ compared with the control and those with other *SlABCB* genes (Fig. 8). In conclusion, in a yeast-based metal sensitivity test assay, the expression of *SlABCB8* and *SlABCB22* compensated the sensitive phenotypes of the mutant strains grown under excess cadmium.

Discussion

In recent years, ABC transporters have become a major focus for research in plants. In such research,154 ABC transporter proteins in total have been appraised, and their members have been divided into eight groups: ABCA–ABCH[31]. Full-size ABCBs are referred as P-glycoprotein (PGP) or multidrug resistance (MDR) proteins. However, the half-size ABCBs have characteristics labeled with names such as the transporter associated with lipid A-like exporter – putative (LLP), the transporter of antigen processing (TAP), and the ABC transporter of mitochondria (ATM)[3]. In tomato, the ABCB subfamily, with 29 members, is the second-largest subfamily, including 8 half-size, 18 full-size, and 3 quarter-size members[6]. The ABCB subfamily of *Arabidopsis* contains at least six full-sized ABCBs, encoding auxin transporters[6]. In our research, genes of the ABCB subfamily with 29 members were identified, including 10 half-size, 16 full-size, and 3 quarter-size members (Fig. 2, Table 2), which is highly consistent with the published data.

Several common and unique motifs in the tomato ABC transporters have been identified through MEME motif analysis. Genes with common motifs were grouped into uniform groups, suggesting that they had similar functions in general[5]. In *O. sativa*, the ABCB subfamily shares four motifs (Motif 1, Motif 2, Motif 4, and Motif 9)[7]. Similar to earlier research, our study revealed that having common motifs often occurs within the same cluster (Fig. 2).

Collinearity analysis revealed a high degree of synteny between tomato and eggplant *ABCB* genes, with 16 collinear pairs identified, suggesting strong evolutionary conservation and potential functional redundancy within the Solanaceae family[22]. Notably, several of the cadmium-responsive *SlABCB* genes identified in our

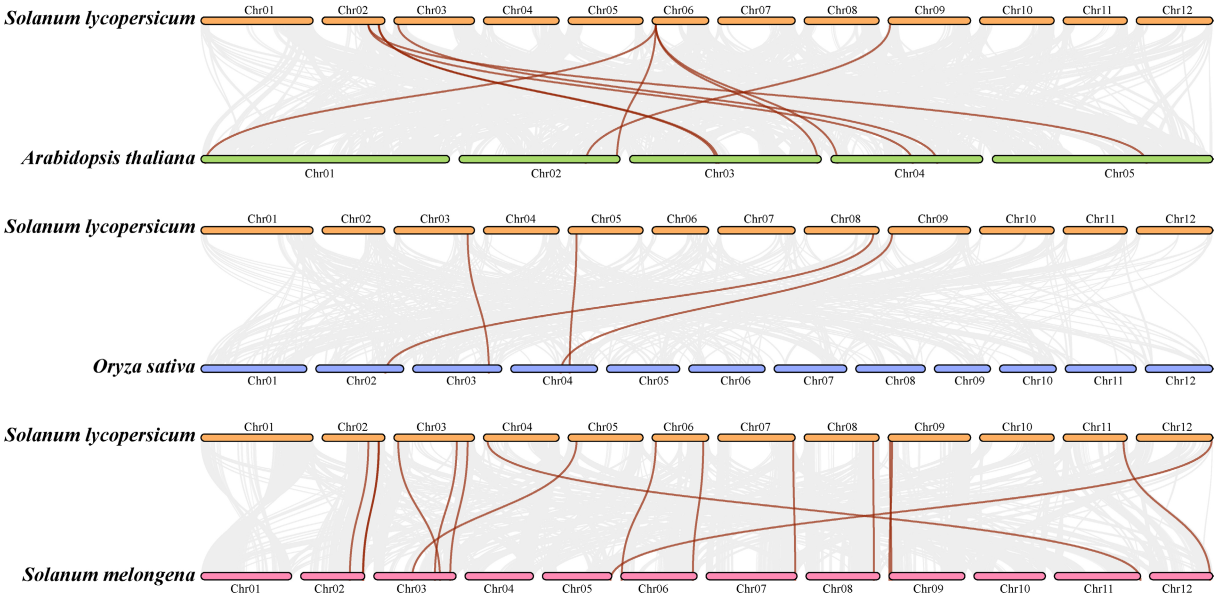

Fig. 4 Collinear correlation of the *ABCB* gene family between *Solanum lycopersicum* L. and (a) *Arabidopsis thaliana*, (b) *Oryza sativa*, and (c) *Solanum melongena*.

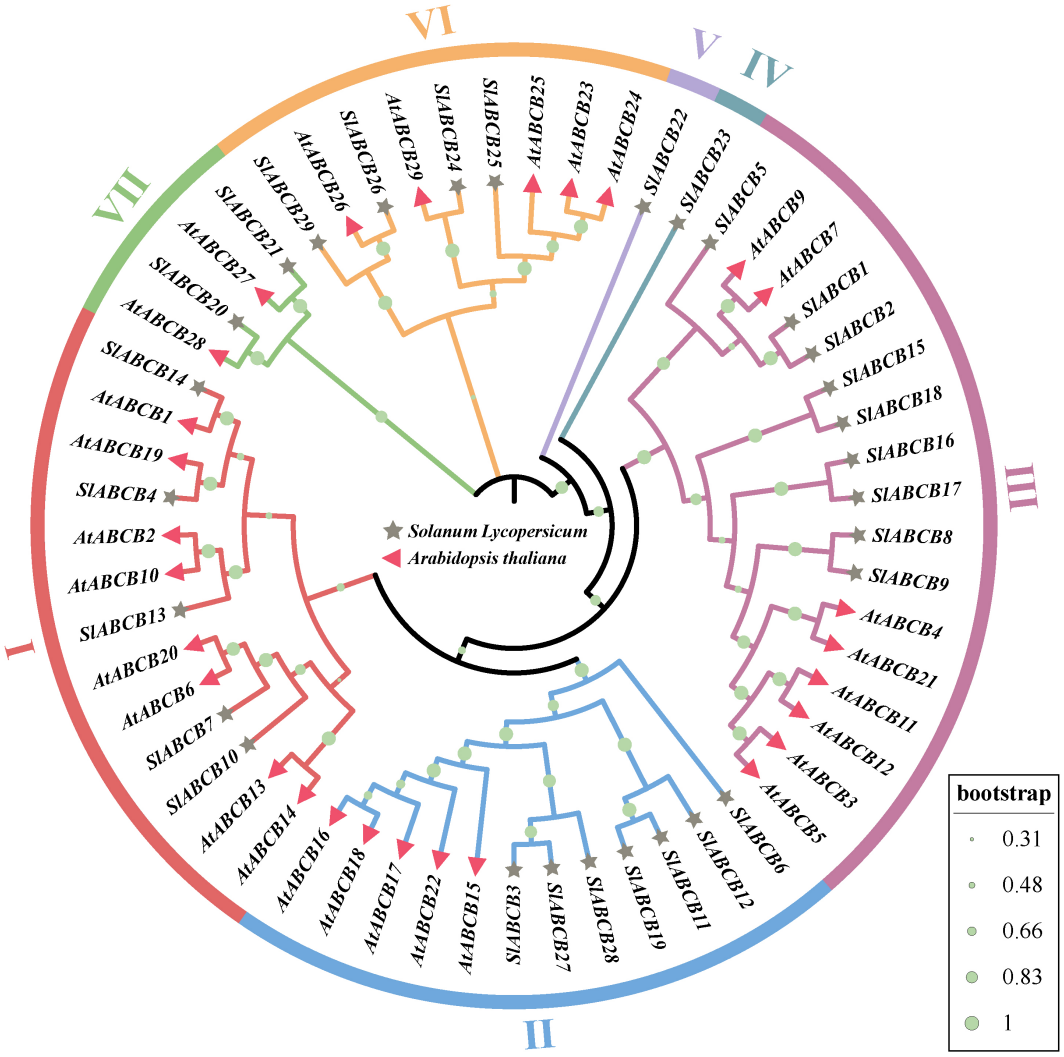

Fig. 5 Phylogenetic tree constructed using MEGA 6.0 by NJ the method based on a total of 58 ABCB proteins comprising 29 *S. lycopersicum* and 29 *Arabidopsis* ABCB proteins. The phylogenetic tree reveals seven main clusters (1–7).

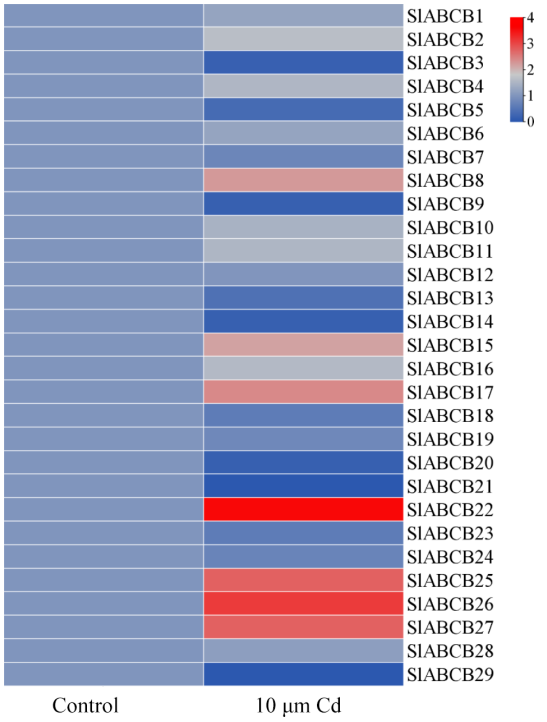

Fig. 6 Heatmap of *SlABCB* genes determined by microarray analysis and RNA-Seq data. The heatmap was generated using TBtools software using the RNA-Seq data.

study, such as *SlABCB8*, *SlABCB15*, *SlABCB22*, *SlABCB25*, *SlABCB26*, and *SlABCB27*, have putative orthologs in the eggplant genome. For instance, according to the collinearity map, *SlABCB22* likely shares a common ancestor with its eggplant counterpart. This high sequence homology implies that these eggplant orthologs may also play roles in heavy metal detoxification, as functional conservation among orthologs across closely related species is frequently observed[6].

An analysis of the member structure of the ABCB subfamily of tomatoes may contribute to functional research. The evolutionary tree revealed that intron–exon arrangements shaped the evolution of this gene family[32,33]. The expression levels of genes with no and few introns are low in plant genomes[34]. Furthermore, the compact gene structure may enable genes to respond rapidly to exogenous or endogenous stimuli in terms of expression[35]. Genes with fewer introns are often associated with rapid transcriptional induction in response to environmental stimuli[35]. This structure allows for quicker mRNA processing and export, which could be crucial for an immediate early response to acute cadmium exposure. Conversely, genes with complex intron–exon architectures (*SlABCB20*, *SlABCB21*, and *SlABCB25*) may be subject to more intricate layers of regulation (Supplementary Fig. S2), including alternative splicing and post-transcriptional modulation[36]. Our genetic structure analysis also indicated that the gene sequences of the *ABCB* subfamily in tomatoes have the same number of exons and introns with similar functional characteristics, which may have emerged during the evolutionary process of repetitive events.

Duplication, genome size, and gene distribution are the main factors influencing the genetic diversity of land plants. The distribution of ABC transporter genes is not uniform throughout the chromosomes, with chromosome 1 and chromosome 4 hosting the largest proportion of ABC transporter members, accounting for 22.43% and 11.21%, respectively[7]. We discovered that chromosome 2 had four *ABCB* transporter genes and that chromosome 3 had six *ABCB* family members (Supplementary Fig. S3). The gene distribution pattern indicated that these genes performed homologous functions in responding to environmental pressure, development, and plant growth[5, 37]. The distribution and mapping of the *ABCB* subfamily members of tomato at the chromosome level will help tomato breeders develop new tomato varieties with ideal traits.

Studies of *cis*-elements can provide a necessary foundation for further functional dissection of the *ABCB* subfamily of genes in tomato. Several hormone-related response elements, including those related to ABA, gibberellin, salicylic acid, MeJA, zein, and auxin responses, were found in the 2,000 bp sequence upstream of the promoter region of the *SlABCB* genes in the prediction analyses. The link between heavy metal stress and hormone signaling is

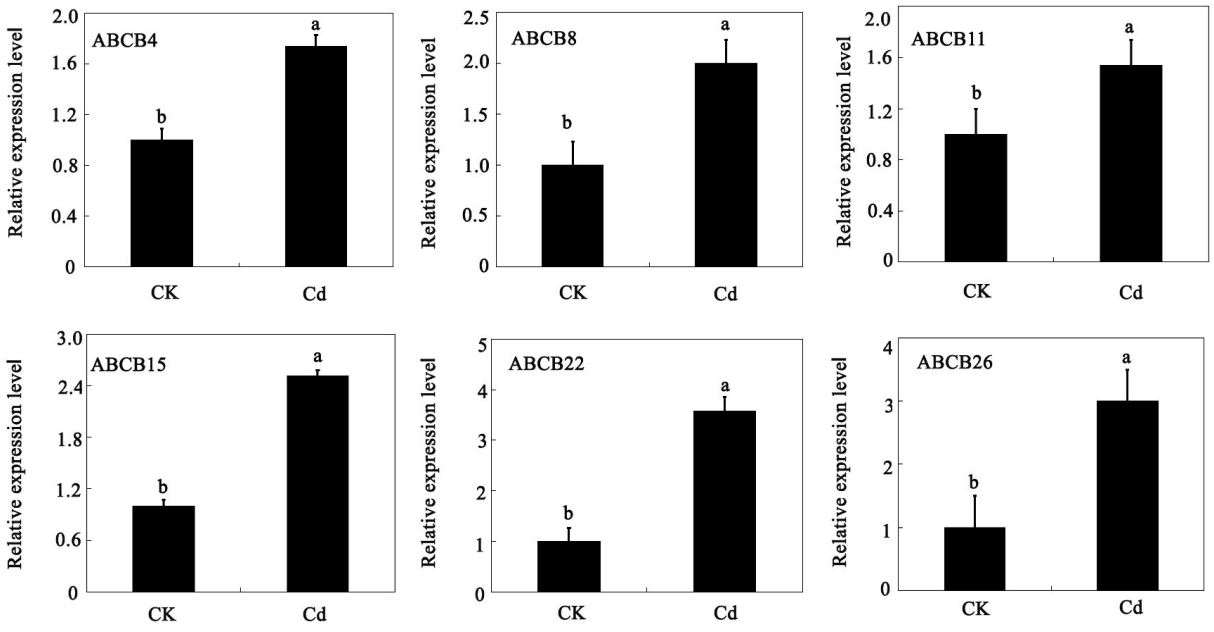

Fig. 7 Expression of *ABCB* genes in tomato under cadmium stress, determined by qRT-PCR. The data in the figure were obtained from three biological replicates ($n = 3$), presented as the means + standard error (SE). Significance differences are denoted by different lowercase letters ($p < 0.05$), according to Duncan's multiple range test.

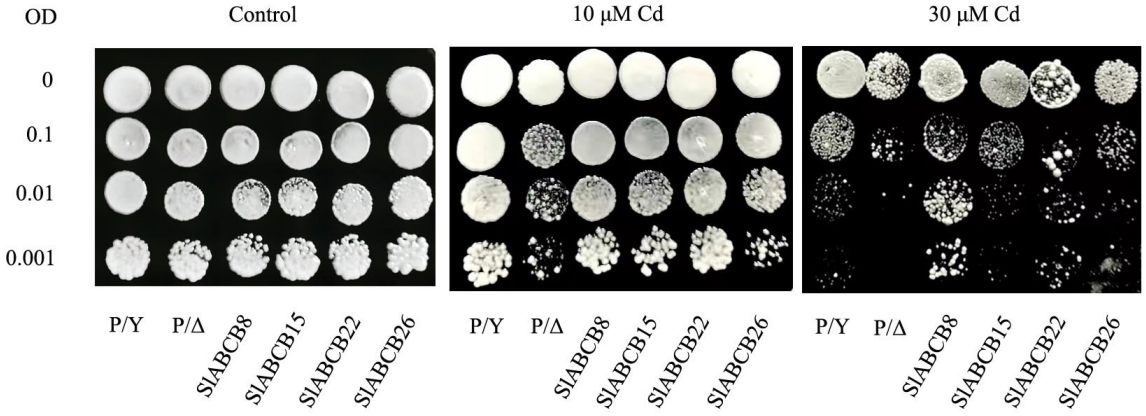

Fig. 8 Functional verification of *SlABCB* genes in yeast. *SlABCB8*, *SlABCB15*, *SlABCB22*, and *SlABCB26* were transformed into Cd-sensitive strains of *Saccharomyces cerevisiae* Δ*yap1* to test their function of mediating tolerance to excess Cd. P/Y, wild-type Y252 transformed with the empty pYES2 vector; P/Δ, the cadmium-sensitive yeast mutant Δ*yap1* transformed with the empty pYES2 vector.

increasingly recognized as a key mechanism in plant adaptation. Our promoter analysis found that many cadmium-responsive *SlABCB* genes contain *cis*-elements for auxin, ABA, jasmonic acid, and salicylic acid (Fig. 2), pointing to likely hormonal regulation. This is particularly relevant for ABCB transporters, many of which are established auxin transporters. Recent work has shown that cadmium stress directly disrupts auxin homeostasis, leading to inhibited root growth[38]. Furthermore, we also found that most of tomato ABCB subfamily promoters contain one or more defense and stress response elements (Fig. 2). The identified Cd-responsive *SlABCB* genes, including *SlABCB8* and *SlABCB22*, may also contribute to the detoxification of other heavy metals like lead or zinc. Evidence from model plants shows that ABC transporters often confer multi-metal resistance. In *Arabidopsis*, *AtABCB25* provides tolerance to both Cd and Zn[39], and numerous rice *OsABC* genes are regulated by diverse heavy metals[37]. The upstream regions of these *SlABCB* genes contain abundant general stress-responsive *cis*-elements alongside metal-specific ones, suggesting that their expression could be coordinated by broad-acting transcription factors responding to various abiotic stresses. Therefore, to functionally validate the role of these predicted *cis*-elements in response to cadmium, future studies could employ luciferase reporter assays with progressive promoter deletions and site-directed mutagenesis of key elements.

As a result of exposure to a wide range of environmental stresses, ABC transporters have undergone positive selective pressures during evolution. According to the phylogenetic tree constructed using the ABCB genomes, these genes seem to have evolved from the same ancestor. A few branches exclusively contained homologous tomato or *Arabidopsis* transporters (Fig. 4). The ABC transporter family members in these gene clusters may perform specific functions in diverse species[39].

Notably, *AmABCB1* was highly expressed in the seeds but not in the leaves. Its expression levels were relatively lower in the pericarps and roots[40]. On the 14th day after pollination (DAP), the expression of *SlABCB4* reached its peak[6]. In the present study, *SlABCB1* showed relatively low expression in leaves and roots, and almost no expression was observed in other tissues and organs. Similarly, the expression level of *SlABCB4* was also at a moderate level in young leaves, flowers, and stems, and its expression peaked at the 3-cm fruit stage (Supplementary Fig. S4). These findings were largely consistent with previous research. According to gene expression data based on the eFP Browser, *SlABCB7*, *SlABCB13*, *SlABCB14*, *SlABCB18*, *SlABCB20*, *SlABCB21*, *SlABCB24*, *SlABCB25*, and *SlABCB29* are widely expressed in all tissues and organs, indicating that they are responsible for basic cell maintenance[6].

Microarray and RNA-Seq data from an earlier study demonstrated that ABC transporter family members are upregulated in response to environmental stress[7]. Following heavy metal (Cd) stress treatment, rice *Os*ABCC9 was upregulated and entered the root vacuoles by chelating Cd, mediating tolerance to and accumulation of Cd[41]. *At*ABCB25 participates in the biosynthesis of molybdenum cofactors and heavy metal tolerance, probably through its function as a glutathione disulfide (GSSG) transporter[42]. The transcriptional level of *ABCG36* in poplar was upregulated under Cd stress. Overexpression of ABCG36 in transgenic *Arabidopsis* enhanced plants' resistance to Cd stress by expelling it from the plants[43]. In this study, *SlABCB22*, *SlABCB26*, and *SlABCB27* were identified as potential cadmium-resistant candidate materials, and were worthy of further study (Fig. 6). Whereas our transcriptome and qRT-PCR analyses identified key *SlABCB* genes that were responsive to cadmium stress at the whole-seedling level, the current study did not separately analyze root versus leaf tissues in detail. Investigating this tissue-specific regulation represents a crucial direction for our future research.

Among the four Cd-tolerant *SlABCB* genes identified in the yeast assay, *SlABCB8* and *SlABCB22* conferred a more pronounced tolerance phenotype compared with *SlABCB15* and *SlABCB26* (Fig. 8). This functional divergence among subfamily members, despite their structural similarities, is a common phenomenon in ABC transporters[9]. Primarily, substrate specificity and transport efficiency are largely determined by the composition of the transmembrane domains (TMDs)[9]. It is plausible that subtle differences in the TMD sequences of *SlABCB8* and *SlABCB22* confer higher binding affinity or transport kinetics for cadmium ions or their complexes compared with *SlABCB15* and *SlABCB26*. This is supported by studies in *Arabidopsis*, where different ABC transporters exhibit distinct roles in cadmium detoxification; for instance, *At*ABCC3 is specifically involved in sequestering Cd–phytochelatin complexes into the vacuoles[44].

The evolutionary history of these transporters across the Solanaceae family warrants further investigation. The recent availability of genomic data for multiple species enables comparative analyses with taxa such as *Capsicum annuum* (bell pepper) and *Solamum tuberosum* (potato)[45]. This study provides a foundational analysis of the ABCB subfamily within *Solanum lycopersicum*. The research offers a very useful reference for future research on the functional divergence and evolution of the ABCB subfamily of genes, especially cadmium transport-related genes.

In conclusion, analyses of gene structure, chromosomal locations, gene duplication, comparative phylogenetics, and *cis*-regulatory

elements were conducted on 29 identified tomato ABCB subfamily genes. Meanwhile, the research offered proof for the biological functions of ABCB subfamily members as transporters in regulating plants' Cd resistance. In addition, it is hoped that this study will have certain reference value for further research on ABC transporter proteins in plants.

Author contributions

The authors confirm contributions to the paper as follows: study design: He G, Liang Q, Yang P, Song G; writing the manuscript: Zhang J; reviewing the manuscript: Gao Y. All the authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

This work was funded by the Hunan Provincial Department of Education Key Project (23A0349), Hunan Province College Students' Innovation Training Program (S202310534072), the Hunan Provincial Natural Science Foundation of China (2025JJ70108), and the Graduate Scientific Research Innovation Project of Hunan Province (CX20231038).

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/vegres-0025-0042)

Dates

Received 12 June 2025; Revised 12 October 2025; Accepted 15 October 2025; Published online 22 December 2025

References

1. Wang P, Chen H, Kopittke PM, Zhao FJ. 2019. Cadmium contamination in agricultural soils of China and the impact on food safety. *Environmental Pollution* 249:1038–48
2. Dahuja A, Kumar RR, Sakhare A, Watts A, Singh B, et al. 2021. Role of ATP-binding cassette transporters in maintaining plant homeostasis under abiotic and biotic stresses. *Physiologica Plantarum* 171:785–801
3. Guo Z, Yuan X, Li L, Zeng M, Yang J, et al. 2022. Genome-wide analysis of the ATP-binding cassette (ABC) transporter family in *Zea mays* L. and its response to heavy metal stresses. *International Journal of Molecular Sciences* 23:2109
4. Do THT, Martinoia E, Lee Y. 2018. Functions of ABC transporters in plant growth and development. *Current Opinion in Plant Biology* 41:32–38
5. Zhang XD, Zhao KX, Yang ZM. 2018. Identification of genomic ATP binding cassette (ABC) transporter genes and Cd-responsive ABCs in *Brassica napus*. *Gene* 664:139–51
6. Ofori PA, Mizuno A, Suzuki M, Martinoia E, Reuscher S, et al. 2018. Genome-wide analysis of ATP binding cassette (ABC) transporters in tomato. *PLoS One* 13:e0200854
7. Qiao Y, Chen ZJ, Liu J, Nan Z, Yang H. 2022. Genome-wide identiffcation of *Oryza sativa*: a new insight for advanced analysis of ABC transporter genes associated with the degradation of four pesticides. *Gene* 834:146613
8. Saha J, Sengupta A, Gupta K, Gupta B. 2015. Molecular phylogenetic study and expression analysis of ATP-binding cassette transporter gene family in *Oryza sativa* in response to salt stress. *Computational Biology and Chemistry* 54:18–32
9. Kang J, Park J, Choi H, Burla B, Kretzschmar T, et al. 2011. Plant ABC transporters. *The Arabidopsis Book* 9:e0153
10. Jenness MK, Carraro N, Pritchard CA, Murphy AS. 2019. The Arabidopsis ATP-binding cassette transporter ABCB21 regulates auxin levels in cotyledons, the root pericycle, and leaves. *Frontiers in Plant Science* 10:806
11. Chen J, Hu Y, Hao P, Tsering T, Xia J, et al. 2023. ABCB-mediated shootward auxin transport feeds into the root clock. *EMBO Reports* 24:e56271
12. Balzan S, Johal GS, Carraro N. 2014. The role of auxin transporters in monocots development. *Frontiers in Plant Science* 5:393
13. Zhu XF, Lei GJ, Wang ZW, Shi YZ, Braam J, et al. 2013. Coordination between apoplastic and symplastic detoxification confers plant aluminum resistance. *Plant Physiology* 162:1947–55
14. Park J, Song WY, Ko D, Eom Y, Hansen TH, et al. 2012. The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. *The Plant Journal* 69:278–88
15. Huang CF, Yamaji N, Mitani N, Yano M, Nagamura Y, et al. 2009. A bacterial-type ABC transporter is involved in aluminum tolerance in rice. *The Plant Cell* 21:655–67
16. Fu S, Lu Y, Zhang X, Yang G, Chao D, et al. 2019. The ABC transporter ABCG36 is reuqired for cadmium tolerance in rice. *Journal of Experimental Botany* 70:5909–18
17. Li H, Li C, Sun D, Yang ZM. 2024. OsPDR20 is an ABCG metal transporter regulating cadmium accumulation in rice. *Journal of Environmental Sciences* 136:21–34
18. El-Gebali S, Mistry J, Bateman A, Eddy SR, Luciani A, et al. 2019. The pfam protein families database in 2019. *Nucleic Acids Research* 47:D427–D432
19. Tordai H, Suhajda E, Sillitoe I, Nair S, Varadi M, et al. 2022. Comprehensive collection and prediction of ABC transmembrane protein structures in the AI era of structural biolgoy. *International Journal of Molecular Sciences* 23:8877
20. Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38:3022–27
21. Nasim J, Malviya N, Kumar R, Yadav D. 2016. Genome-wide bioinformatics analysis of Dof transcription factor gene family of chickpea and its comparative phylogenetic assessment with Arabidopsis and rice. *Plant Systematics and Evolution* 302:1009–26
22. Wang Y, Tang H, Debarry JD, Tan X, Li J, et al. 2012. *MCScanX*: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Research* 40:e49
23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35:1547–49
24. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, et al. 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Molecular Plant* 13:1194–202
25. Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, et al. 2002. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Research* 30:325–27
26. Xue M, Zhou Y, Yang Z, Lin B, Yuan J, et al. 2014. Comparisons in subcellular and biochemical behaviors of cadmium between low-Cd and high-Cd accumulation cultivars of pakchoi (*Brassica chinensis* L.). *Frontiers of Environmental Science & Engineering* 8:226–38
27. Zhou Q, Guo JJ, He CT, Shen C, Huang YY, et al. 2016. Comparative transcriptome analysis between low- and high-cadmium-accumulating genotypes of pakchoi (*Brassica chinensis* L.) in response to cadmium stress. *Environmental Science & Technology* 50:6485–94
28. Feng SJ, Liu XS, Tao H, Tan SK, Chu SS, et al. 2016. Variation of DNA methylation patterns associated with gene expression in rice (*Oryza sativa*) exposed to cadmium. *Plant, Cell & Environment* 39:2629–49
29. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–8

30. Elble R. 1992. A simple and efficient procedure for transformation of yeasts. *BioTechniques* 13:18–20
31. Khan KA. 2022. Genome wide analysis of ATP-binding Cassette (ABC) transporter in the eastern honey bee (*Apis cerana* Fabricius, 1793). *Journal of King Saud University – Science* 34:101766
32. Flagel LE, Wendel JF. 2009. Gene duplication and evolutionary novelty in plants. *New Phytologist* 183:557–64
33. Moore RC, Purugganan MD. 2005. The evolutionary dynamics of plant duplicate genes. *Current Opinion in Plant Biology* 8:122–28
34. Mattick JS, Gagen MJ. 2001. The evolution of controlled multitasked gene networks: the role of introns and other noncoding RNAs in the development of complex organisms. *Molecular Biology and Evolution* 18:1611–30
35. Jeffares DC, Penkett CJ, Bähler J. 2008. Rapidly regulated genes are intron poor. *Trends in Genetics* 24:375–78
36. Chaudhary S, Khokhar W, Jabre I, Reddy ASN, Byrne LJ, et al. 2019. Alternative splicing and protein diversity: plants versus animals. *Frontiers in Plant Science* 10:708
37. Afzal Malik W, Afzal M, Chen X, Cui R, Lu X, et al. 2022. Systematic analysis and comparison of ABC proteins superfamily confer structural, functional and evolutionary insights into four cotton species. *Industrial Crops and Products* 177:114433
38. Feng S, Li N, Chen H, Liu Z, Li C, et al. 2024. Large-scale analysis of the ARF and Aux/IAA gene families in 406 horticultural and other plants. *Molecular Horticulture* 4:13
39. Nguyen VNT, Moon S, Jung KH. 2014. Genome-wide expression analysis of rice ABC transporter family across spatio-temporal samples and in response to abiotic stresses. *Journal of Plant Physiology* 171:1276–88
40. Loza-Muller L, Shitan N, Yamada Y, Vázquez-Flota F. 2021. AmABCB1, an alkaloid transporter from seeds of *Argemone mexicana* L (Papaveraceae). *Planta* 254:122
41. Yang G, Fu S, Huang J, Li L, Long Y, et al. 2021. The tonoplast-localized transporter OsABCC9 is involved in cadmium tolerance and accumulation in rice. *Plant Science* 307:110894
42. Schaedler TA, Thornton JD, Kruse I, Schwarzländer M, Meyer AJ, et al. 2014. A conserved mitochondrial ATP-binding cassette transporter exports glutathione polysulfide for cytosolic metal cofactor assembly. *Journal of Biological Chemistry* 289:23264–74
43. Wang H, Liu Y, Peng Z, Li J, Huang W, et al. 2019b. Ectopic expression of Poplar ABC transporter PtoACBG36 confers Cd tolerance in *Arabidopsis thaliana*. *International Journal of Molecular Sciences* 20:3293–305
44. Brunetti P, Zanella L, De Paolis A, Di Litta D, Cecchetti V, et al. 2015. Cadmium-inducible expression of the ABC-type transporter *AtABCC3* increases phytochelatin-mediated cadmium tolerance in *Arabidopsis*. *Journal of Experimental Botany* 66:3815–29
45. Liu Z, Shen S, Li C, Zhang C, Chen X, et al. 2025. SolR: a comprehensive Solanaceae information resource for comparative and functional genomic study. *Nucleic Acids Research* 53:D1623–D1632