

RESEARCH ARTICLE

Genome-wide analysis of ATP binding cassette (ABC) transporters in tomato

Peter Amoako Ofori¹*, Ayaka Mizuno¹*, Mami Suzuki¹, Enrico Martinoia², Stefan Reuscher¹, Koh Aoki³, Daisuke Shibata⁴, Shungo Otagaki¹, Shogo Matsumoto¹, Katsuhiko Shiratake^{1*}

1 Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, **2** Department of Plant and Microbial Biology, University of Zurich, Zurich, Switzerland, **3** Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Japan, **4** Kazusa DNA Research Institute, Kisarazu, Japan

* These authors contributed equally to this work.

* shira@agr.nagoya-u.ac.jp



OPEN ACCESS

Citation: Ofori PA, Mizuno A, Suzuki M, Martinoia E, Reuscher S, Aoki K, et al. (2018) Genome-wide analysis of ATP binding cassette (ABC) transporters in tomato. PLoS ONE 13(7): e0200854. <https://doi.org/10.1371/journal.pone.0200854>

Editor: Kentaro Yano, Meiji Daigaku - Ikuta Campus, JAPAN

Received: May 16, 2018

Accepted: July 3, 2018

Published: July 26, 2018

Copyright: © 2018 Ofori et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported partially by the Programme for Promotion of Basic and Applied Researchers for Innovations from Bio-oriented Industry from the Bio-oriented Technology Research Advancement Institution (BRAINI), the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry from the Ministry of Agriculture,

Abstract

ATP binding cassette (ABC) transporters are proteins that actively mediate the transport of a wide range of molecules, such as organic acids, metal ions, phytohormones and secondary metabolites. Therefore, ABC transporters must play indispensable roles in growth and development of tomato, including fruit development. Most ABC transporters have transmembrane domains (TMDs) and belong to the ABC protein family, which includes not only ABC transporters but also soluble ABC proteins lacking TMDs. In this study, we performed a genome-wide identification and expression analysis of genes encoding ABC proteins in tomato (*Solanum lycopersicum*), which is a valuable horticultural crop and a model plant for studying fleshy fruits. In the tomato genome, a total of 154 genes putatively encoding ABC transporters, including 9 ABCAs, 29 ABCBs, 26 ABCCs, 2 ABCDs, 2 ABCEs, 6 ABCFs, 70 ABCGs and 10 ABCIs, were identified. Gene expression data from the eFP Browser and reverse transcription-semi-quantitative PCR analysis revealed their tissue-specific and development-specific expression profiles. This work suggests physiological roles of ABC transporters in tomato and provides fundamental information for future studies of ABC transporters not only in tomato but also in other Solanaceae species.

Introduction

ATP binding cassette (ABC) proteins are proteins harboring an ATP binding domain, called nucleotide binding domain or fold (NBD/NBF), which contains highly conserved motifs, such as the Walker A and Walker B motifs, the ABC signature, the H loop and the Q loop [1]. ABC proteins are universally found in all organisms, including fungi, plants and animals [2]. Some members of the ABC proteins are soluble proteins and do not contain any transmembrane domain (TMD). The ABC proteins harboring TMDs are called ABC transporters and function as ATP-driven primary transporters for active transport of various molecules [3]. A typical functional ABC transporter contains 2 NBDs and 2 TMDs. The two NBDs synergistically bind and hydrolyze ATP to generate energy, which eventually causes conformational changes in the

Forestry and Fisheries (MAFF), the Cross-ministerial Strategic Innovation Promotion Program (SIP) from the Cabinet Office, and the Grant-in-Aids for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS).

Competing interests: The authors have declared that no competing interests exist.

TMDs to create a pore for substrate transport, while the TMDs serve as a pathway for unidirectional transport of the substrate [1]. ABC transporters harboring two TMDs and two NBDs are called full-size ABC transporters. On the other hand, ABC transporters harboring only one TMD and one NBD are called half-size. ABC transporters encoded by four genes, two for TMDs and two for NBDs are so-called quarter-size ABC transporters [3,4].

ABC transporters are grouped into eight subfamilies, namely ABCA to ABCI. Plants do not have any ABCH subfamily. Generally, plants possess twice as many as ABC transporters as not in animals. It is assumed that this is due to the sessile nature of plants for growing under various biotic and abiotic stresses [5]. ABC transporters of plants are engaged in numerous functions, including secondary metabolite transport [6,7], heavy metal detoxification [8], antibiotic transport [9] and phytohormone transport [10,11]. ABC transporter counterparts in animal are also shown to function as ion channels, channel regulators [12,13] and in protein targeting [14].

A genome-wide analysis is the comprehensive identification of all genes of the respective family including their family members and organization of their information. This approach provides essential information, such as evolutionary history, diversity and relationship among genes and proteins, which serves as useful fundamental resources for further investigations. Genome-wide analyses of ABC transporters in *Arabidopsis* [15], rice [16], maize [17], *Lotus japonicus* [18], grape [19], pineapple [20], and *Hevea brasiliensis* [4] have already been performed. Whereas little is known about ABC transporters in Solanaceae, including tomato.

Tomato is an important vegetable crop and is often used as a model plant for studying developmental physiology of fleshy fruits recently. The advantages of tomato in research are the availability of its high quality whole genome sequencing data (Sol Genomics Network (SGN), <https://solgenomics.net/>) [21], expressed sequence tag (EST) database (TomatEST, http://biosrv.cab.unina.it/tomatestdb/transcript_browser.html) [22] and full-length cDNA resources (TOMATOMICS: <http://plantomics.mind.meiji.ac.jp/tomatomics/>) [23,24]. Transcriptome databases at Tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi) [25,26] and SGN-TEA (<http://tea.solgenomics.net/>) [27] and metabolome database at MoTo DB (<http://www.transplantdb.eu/node/1843>) [28] are also available for tomato. Micro-Tom is a dwarf tomato variety and an excellent tool for genetic and physiological studies of fruit development and physiology, because of its small size and short lifecycle [29].

In this study, a genome wide analysis was performed to provide information of ABC proteins in tomato. A total of 154 genes putatively encoding ABC proteins were identified in tomato genome. Among these ABC proteins, 47 proteins are soluble ABC proteins lacking any TMDs, while 107 proteins contain TMDs and they are considered to function as ABC transporters. Phylogenetic analysis revealed the evolutionary relationships of tomato ABC proteins. In addition, protein structure, in silico and reverse transcription-semi-quantitative PCR gene expression analyses were performed to provide fundamental information for further ABC protein studies not only in tomato but also in other Solanaceae species.

Materials and methods

Identification of ABC proteins in tomato

The BLAST tool of Sol Genomics Network (SGN, <http://www.solgenomics.net/>) [21] was used for genome-wide identification of genes encoding ABC proteins in tomato. Known ABC proteins of tomato reported by Andolfo et al. [30] and some members of the *Arabidopsis* ABC subfamilies [15] were used as queries for BLAST search in the tomato genome (SL3.0 and ITAG3.10) [26]. Identified proteins with at least 30% similarity to the query sequence or E-value less than E-20 were selected. Presence of ABC signature, Walker A and Walker B motifs

was confirmed by using the Conserved Domain Database of NCBI (<https://www.ncbi.nlm.nih.gov/cdd/>) [31]. The predicted genes encoding ABC proteins from SL3.0 of SGN were confirmed by comparing with another tomato genome database TMCSv1.2.1 from TOMA-TOMICS (<http://plantomics.mind.meiji.ac.jp/tomatomics/download.php>) [23,24].

Phylogenetic, in silico gene expression and protein structure analyses. Phylogenetic analysis was conducted to classify the identified ABC proteins into their respective subfamilies. Entire protein sequences of ABC proteins were aligned using the multiple sequence alignment tool of ClustalW program (<http://www.genome.jp/tools/clustalw/>) [32] and subjected to cluster analysis by the distance with the neighbor-joining method using MEGA6.06 software (Molecular Evolutionary Genetics Analysis, <https://www.megasoftware.net/>) [33]. Gene expression data of ABC proteins in various tomato tissues were obtained from the Tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi) [25,26]. The Pfam web server (<http://pfam.xfam.org/>) [34] was used to characterize the topology of ABC proteins comprising TMD and NBD.

Plant materials. Tomato (*Solanum lycopersicum*) 'Micro-Tom' was used for gene expression analysis. The Micro-Tom strain used in this study was obtained from the National Biore-source Project (NBRP)-Tomato (<http://tomato.nbrp.jp/browseSearchEn.html>) with an accession number TOMJPF00001. Plants were grown in growth chamber (Biotron LPH-350S, NK Systems) adjusted to 25°C, 16 h light/8 h dark period and 60% relative humidity. Tap water was supplied twice a week. Half concentration of Otsuka liquid fertilizer (Otsuka Chemicals Co., Ltd.) was applied weekly. Young and mature leaves, root, stem, flower, developing fruit tissues at 3, 7, 14, 21, 28 days after pollination (DAP), breaker, orange and red stages were sampled, frozen in liquid nitrogen and stored at -80°C.

RNA extraction and RT-semi-quantitative PCR (RT-sqPCR) expression analysis. Extraction of total RNA from developing fruits at 14 and 21 DAP was performed using the RNA Suisui-R kit (Rizo). RNA of other tissues was isolated using TRIzol reagent (Life Technologies). PrimeScript RT reagent kit (Takara) was used to synthesize the cDNA. RT-sqPCR was conducted using SYBR Premix Ex Taq kit (Takara) and the ubiquitin gene, *SIUBQ* (*Solyc01g056940*) was used as an internal control. Primer sequences and PCR conditions are shown in [S1 Table](#).

Results and discussion

Genome-wide identification of ABC proteins in tomato

To clarify the gene family of ABC proteins in tomato, BLAST search on tomato genome database Sol Genomics Network (SGN, <http://www.solgenomics.net/>) [21] was performed. We searched all the tomato ABC proteins using SL3.0 of SGN database. As a result, 154 genes potentially encoding ABC proteins were found ([Table 1](#)). Phylogenetic analysis of the tomato ABC proteins was performed and the obtained phylogenetic tree is shown in [Fig 1](#).

In a previous study, Andolfo et al. [30] identified 180 ABC proteins in the tomato genome, while we found 154 ABC proteins. So we compared non-overlapping candidates between our study and Andolfo et al. [30] ([S2 Table](#)). In this study, 3 non-overlapping putative tomato ABC proteins were identified whereas 29 ABC proteins were identified only in Andolfo et al. [30] ([S2 Table](#)). All the 3 ABC proteins identified in this study have NBDs. On the other hand, the 29 ABC proteins found only in Andolfo et al. [30] have no NBD. Thus, we concluded that the 29 candidates without NBD in Andolfo et al. [30] are not ABC proteins and may be mispredicted. Therefore, we did not include them in our list ([Table 1](#)).

In addition, since some of the genes may not be computationally annotated in SL3.0 of SGN database, we confirmed the gene prediction of SL3.0 by comparing this database with

Table 1. Inventory of tomato ABC proteins with their in silico gene expression profiles.

Sub-family	Gene name	Locus	Size (AA)	Best hit EST	Topology	Old name	Expression												Abs value
							L	R	B	F	F1	F2	F3	M	Bk	Rd			
ABCA	SIABCA1	<i>Solyc04g015970.2</i>	1,910	SGN-E342230	(TMD-NBD)×2														26.7
	SIABCA2	<i>Solyc03g113040.2</i>	946	SGN-E1283433	TMD-NBD														10.9
	SIABCA3	<i>Solyc03g113060.2</i>	945	SGN-E1279186	TMD-NBD														41.1
	SIABCA4	<i>Solyc06g070920.2</i>	639	SGN-E547444	TMD-NBD														31.0
	SIABCA5	<i>Solyc06g070940.2</i>	944	SGN-E373720	TMD-NBD														21.7
	SIABCA6	<i>Solyc06g070950.1</i>	892	-	TMD-NBD														0.85
	SIABCA7	<i>Solyc06g070960.1</i>	927	-	TMD-NBD														0.57
	SIABCA8	<i>Solyc03g113080.2</i>	359	-	NBD														13.8
	SIABCA9	<i>Solyc03g113070.2</i>	577	-	NBD														11.7
ABCB	SIABCB1	<i>Solyc02g071340.1</i>	1,264	-	(TMD-NBD)×2														0.04
	SIABCB2	<i>Solyc02g071350.2</i>	1,264	-	(TMD-NBD)×2														1.89
	SIABCB3	<i>Solyc02g087410.2</i>	1,263	-	(TMD-NBD)×2														5.79
	SIABCB4	<i>Solyc02g087870.2</i>	1,250	SGN-E701700	(TMD-NBD)×2	SIMDR1													26.6
	SIABCB5	<i>Solyc03g005860.2</i>	1,260	-	(TMD-NBD)×2														0.14
	SIABCB6	<i>Solyc03g093650.2</i>	1,228	-	(TMD-NBD)×2														3.95
	SIABCB7	<i>Solyc04g010310.2</i>	1,286	SGN-E1249810	(TMD-NBD)×2														20.7
	SIABCB8	<i>Solyc06g009280.1</i>	1,290	-	(TMD-NBD)×2														5.56
	SIABCB9	<i>Solyc06g009290.2</i>	1,401	SGN-E550470	(TMD-NBD)×2	SIMDR2													47.3
	SIABCB10	<i>Solyc06g072960.1</i>	1,029	-	(TMD-NBD)×2														0.02
	SIABCB11	<i>Solyc07g018130.1</i>	1,276	-	(TMD-NBD)×2														0.04
	SIABCB12	<i>Solyc07g064120.1</i>	1,260	-	(TMD-NBD)×2														0.39
	SIABCB13	<i>Solyc08g076720.2</i>	1,258	SGN-E228826	(TMD-NBD)×2														25.8
	SIABCB14	<i>Solyc09g008240.2</i>	1,315	SGN-E315464	(TMD-NBD)×2														52.1
	SIABCB15	<i>Solyc11g067310.1</i>	1,290	-	(TMD-NBD)×2														0.61
	SIABCB16	<i>Solyc12g098840.1</i>	1,281	-	(TMD-NBD)×2														25.4
	SIABCB17	<i>Solyc12g098870.1</i>	1,313	-	(TMD-NBD)×2														2.89
	SIABCB18	<i>Solyc11g067300.1</i>	1,261	-	TMD-TMD-NBD-TMD-NBD														3.16
	SIABCB19	<i>Solyc05g013890.1</i>	955	-	NBD-TMD-NBD														0.28
	SIABCB20	<i>Solyc03g026310.2</i>	664	SGN-E1284789	TMD-NBD														32.4
	SIABCB21	<i>Solyc03g114950.2</i>	639	SGN-E746787	TMD-NBD														57.7
	SIABCB22	<i>Solyc03g122050.1</i>	673	-	TMD-NBD														0.20
	SIABCB23	<i>Solyc03g122070.1</i>	667	-	TMD-NBD														0.49
	SIABCB24	<i>Solyc09g009910.2</i>	640	SGN-E1285685	TMD-NBD														3.52
	SIABCB25	<i>Solyc09g055350.2</i>	726	SGN-E1270076	TMD-NBD														28.2
	SIABCB26	<i>Solyc00g304030.1</i>	1,081	-	NBD-TMD														0.00
	SIABCB27	<i>Solyc12g049120.1</i>	349	-	NBD														0.00
	SIABCB28	<i>Solyc12g049130.1</i>	108	-	NBD														0.00
	SIABCB29	<i>Solyc12g070280.1</i>	232	SGN-E276294	NBD														53.8
ABCC	SIABCC1	<i>Solyc01g080640.2</i>	1,499	SGN-E701199	(TMD-NBD)×2													116	
	SIABCC2	<i>Solyc03g007530.2</i>	1,468	SGN-E313421	(TMD-NBD)×2													15.2	
	SIABCC3	<i>Solyc03g117540.2</i>	1,482	SGN-E230070	(TMD-NBD)×2													6.79	
	SIABCC4	<i>Solyc06g036490.1</i>	1,194	-	(TMD-NBD)×2													0.02	
	SIABCC5	<i>Solyc07g065320.2</i>	1,506	SGN-E206721	(TMD-NBD)×2													8.11	
	SIABCC6	<i>Solyc08g006880.2</i>	1,627	SGN-E303088	(TMD-NBD)×2	SIMRP2												114	
	SIABCC7	<i>Solyc08g081890.2</i>	1,480	SGN-E1281130	(TMD-NBD)×2													28.6	
	SIABCC8	<i>Solyc09g064440.2</i>	1,532	SGN-E1307571	(TMD-NBD)×2													23.3	

(Continued)

Table 1. (Continued)

Sub-family	Gene name	Locus	Size (AA)	Best hit EST	Topology	Old name	Expression												Abs value
							L	R	B	F	F1	F2	F3	M	Bk	Rd			
SLABCC	SLABCC9	<i>Solyc09g075020.2</i>	1,514	SGN-E345495	(TMD-NBD)×2													66.0	
	SLABCC10	<i>Solyc10g019270.1</i>	1,220	-	(TMD-NBD)×2													3.39	
	SLABCC11	<i>Solyc10g024420.1</i>	1,478	SGN-E128420	(TMD-NBD)×2	SIMRP1												312	
	SLABCC12	<i>Solyc12g044820.1</i>	1,459	SGN-E689095	(TMD-NBD)×2													53.8	
	SLABCC13	<i>Solyc05g014380.2</i>	1,136	SGN-E1256841	TMD-NBD-TMD	SIMRP3												69.1	
	SLABCC14	<i>Solyc00g283010.1</i>	646	-	TMD-NBD													1.38	
	SLABCC15	<i>Solyc11g065710.1</i>	773	-	TMD-NBD													1.33	
	SLABCC16	<i>Solyc11g065720.1</i>	652	-	TMD-NBD													1.65	
	SLABCC17	<i>Solyc12g036150.1</i>	374	SGN-E213562	TMD-NBD													11.4	
	SLABCC18	<i>Solyc12g036140.1</i>	486	-	NBD-TMD													13.1	
	SLABCC19	<i>Solyc02g044000.1</i>	604	-	NBD													14.0	
	SLABCC20	<i>Solyc02g044050.1</i>	492	-	NBD													7.49	
	SLABCC21	<i>Solyc05g014390.2</i>	282	-	NBD													83.5	
	SLABCC22	<i>Solyc05g014500.1</i>	90	-	NBD													22.7	
	SLABCC23	<i>Solyc06g036480.1</i>	135	-	NBD													0.11	
	SLABCC24	<i>Solyc10g019280.1</i>	54	-	NBD													0.00	
SLABCC25	<i>Solyc12g036160.1</i>	233	-	NBD													22.0		
SLABCC26	<i>Solyc12g044810.1</i>	166	-	NBD													16.2		
ABCD	SIABCD1	<i>Solyc04g055120.2</i>	1,345	SGN-E707100	(TMD-NBD)×2												37.9		
	SIABCD2	<i>Solyc12g017420.1</i>	706	SGN-E1282388	TMD-NBD												27.8		
ABCE	SIABCE1	<i>Solyc07g008340.2</i>	579	SGN-E745894	NBD-NBD												79.6		
	SIABCE2	<i>Solyc08g075360.1</i>	607	-	NBD-NBD												5.04		
ABCF	SIABCF1	<i>Solyc04g051800.2</i>	696	SGN-E738084	NBD-NBD												296		
	SIABCF2	<i>Solyc06g074940.2</i>	575	SGN-E745759	NBD-NBD												33.7		
	SIABCF3	<i>Solyc07g008610.1</i>	696	SGN-E1284822	NBD-NBD												65.7		
	SIABCF4	<i>Solyc08g082850.2</i>	717	SGN-E745898	NBD-NBD												131		
	SIABCF5	<i>Solyc10g012190.1</i>	688	-	NBD-NBD												0.12		
	SIABCF6	<i>Solyc11g069090.1</i>	602	SGN-E717588	NBD-NBD												1,277		
ABCG	SIABCG1	<i>Solyc01g006720.2</i>	725	SGN-E539555	NBD-TMD												34.3		
	SIABCG2	<i>Solyc01g097430.2</i>	839	-	NBD-TMD												12.7		
	SIABCG3	<i>Solyc01g105450.2</i>	628	SGN-E320849	NBD-TMD												19.7		
	SIABCG4	<i>Solyc03g007690.1</i>	598	SGN-E711119	NBD-TMD												42.2		
	SIABCG5	<i>Solyc03g019760.2</i>	711	SGN-E345650	NBD-TMD												28.8		
	SIABCG6	<i>Solyc03g113690.1</i>	659	-	NBD-TMD												1.06		
	SIABCG7	<i>Solyc04g006960.2</i>	676	SGN-E205662	NBD-TMD	SIWBC8											4.69		
	SIABCG8	<i>Solyc04g010200.1</i>	719	SGN-E1278817	NBD-TMD	SIWBC4											24.5		
	SIABCG9	<i>Solyc04g010210.1</i>	715	SGN-E1282871	NBD-TMD	SIWBC5											4.28		
	SIABCG10	<i>Solyc04g070970.2</i>	723	SGN-E328516	NBD-TMD												43.3		
	SIABCG11	<i>Solyc05g008350.2</i>	711	SGN-E730349	NBD-TMD	SIWBC10											43.8		
	SIABCG12	<i>Solyc05g051530.2</i>	531	SGN-E349400	NBD-TMD	SIWBC7											47.9		
	SIABCG13	<i>Solyc05g054890.2</i>	751	SGN-E1255617	NBD-TMD	SIWBC3											6.54		
SIABCG14	<i>Solyc05g056470.1</i>	615	-	NBD-TMD												9.74			
SIABCG15	<i>Solyc06g072090.1</i>	661	-	NBD-TMD												0.47			
SIABCG16	<i>Solyc06g072100.1</i>	716	-	NBD-TMD												0.48			
SIABCG17	<i>Solyc06g074970.1</i>	603	SGN-E1260065	NBD-TMD	SIWBC6											11.5			
SIABCG18	<i>Solyc07g053300.1</i>	609	-	NBD-TMD												1.07			
SIABCG19	<i>Solyc07g062630.1</i>	622	-	NBD-TMD												0.28			

(Continued)

Table 1. (Continued)

Sub-family	Gene name	Locus	Size (AA)	Best hit EST	Topology	Old name	Expression											Abs value
							L	R	B	F	F1	F2	F3	M	Bk	Rd		
	SIABCG20	<i>Solyc07g063400.2</i>	614	-	NBD-TMD													5.50
	SIABCG21	<i>Solyc08g005580.2</i>	656	SGN-E211225	NBD-TMD													4.26
	SIABCG22	<i>Solyc08g075430.2</i>	647	SGN-E706558	NBD-TMD	SIWBC2												41.2
	SIABCG23	<i>Solyc09g005970.1</i>	739	SGN-E379457	NBD-TMD													3.11
	SIABCG24	<i>Solyc09g098410.1</i>	730	-	NBD-TMD													0.00
	SIABCG25	<i>Solyc11g009100.1</i>	650	SGN-E218423	NBD-TMD													31.3
	SIABCG26	<i>Solyc11g065350.1</i>	683	-	NBD-TMD													44.5
	SIABCG27	<i>Solyc11g065360.1</i>	689	-	NBD-TMD													5.93
	SIABCG28	<i>Solyc11g069710.1</i>	724	SGN-E1306745	NBD-TMD	SIWBC1												17.0
	SIABCG29	<i>Solyc12g013630.1</i>	629	-	NBD-TMD													12.4
	SIABCG30	<i>Solyc12g013640.1</i>	631	-	NBD-TMD													0.23
	SIABCG31	<i>Solyc12g019620.1</i>	838	SGN-E1245045	NBD-TMD	SIPDR2												1.08
	SIABCG32	<i>Solyc12g019640.1</i>	609	-	NBD-TMD													2.16
	SIABCG33	<i>Solyc01g101070.2</i>	1,448	SGN-E542052	(NBD-TMD)×2													11.8
	SIABCG34	<i>Solyc02g081870.2</i>	1,402	-	(NBD-TMD)×2													0.02
	SIABCG35	<i>Solyc03g120980.2</i>	1,501	SGN-E128965	(NBD-TMD)×2													93.8
	SIABCG36	<i>Solyc05g018510.2</i>	1,422	SGN-E699701	(NBD-TMD)×2	SIPDR1												43.2
	SIABCG37	<i>Solyc05g053570.2</i>	1,411	-	(NBD-TMD)×2													10.3
	SIABCG38	<i>Solyc05g053590.2</i>	1,413	-	(NBD-TMD)×2													50.1
	SIABCG39	<i>Solyc05g053600.2</i>	1,413	SGN-E1300502	(NBD-TMD)×2													16.6
	SIABCG40	<i>Solyc05g053610.2</i>	1,426	SGN-E357332	(NBD-TMD)×2													174
	SIABCG41	<i>Solyc05g055330.2</i>	1,479	-	(NBD-TMD)×2													18.5
	SIABCG42	<i>Solyc06g065670.2</i>	1,409	SGN-E546084	(NBD-TMD)×2													12.1
	SIABCG43	<i>Solyc06g076930.1</i>	1,426	SGN-E243451	(NBD-TMD)×2													20.7
	SIABCG44	<i>Solyc08g067610.2</i>	1,455	SGN-E1249186	(NBD-TMD)×2													40.8
	SIABCG45	<i>Solyc08g067620.2</i>	1,454	-	(NBD-TMD)×2													18.5
	SIABCG46	<i>Solyc09g091660.2</i>	1,441	SGN-E541199	(NBD-TMD)×2													80.6
	SIABCG47	<i>Solyc09g091670.2</i>	1,429	SGN-E356859	(NBD-TMD)×2													16.1
	SIABCG48	<i>Solyc11g007280.1</i>	1,469	-	(NBD-TMD)×2													0.03
	SIABCG49	<i>Solyc11g007290.1</i>	1,468	-	(NBD-TMD)×2													0.22
	SIABCG50	<i>Solyc11g007300.1</i>	1,465	-	(NBD-TMD)×2													0.01
	SIABCG51	<i>Solyc11g067000.1</i>	1,464	-	(NBD-TMD)×2													9.49
	SIABCG52	<i>Solyc12g098210.1</i>	1,426	-	(NBD-TMD)×2													0.63
	SIABCG53	<i>Solyc12g100180.1</i>	1,436	SGN-E546066	(NBD-TMD)×2													57.2
	SIABCG54	<i>Solyc12g100190.1</i>	1,429	-	(NBD-TMD)×2													13.5
	SIABCG55	<i>Solyc00g233480.1</i>	184	-	NBD													45.6
	SIABCG56	<i>Solyc01g105400.2</i>	117	SGN-E218425	NBD													0.75
	SIABCG57	<i>Solyc04g025170.2</i>	1,021	SGN-E286554	NBD													16.6
	SIABCG58	<i>Solyc05g051540.1</i>	131	-	NBD													12.6
	SIABCG59	<i>Solyc06g036240.1</i>	641	-	NBD													0.59
	SIABCG60	<i>Solyc06g075020.2</i>	1,095	-	NBD													2.26
	SIABCG61	<i>Solyc07g065770.2</i>	227	SGN-E327102	NBD													6.66
	SIABCG62	<i>Solyc09g008000.2</i>	1,092	SGN-E330243	NBD													9.86
	SIABCG63	<i>Solyc11g018690.1</i>	343	SGN-E1293717	NBD	SIWBC9, 11												18.7
	SIABCG64	<i>Solyc11g069820.1</i>	1,094	-	NBD													2.63

(Continued)

Table 1. (Continued)

Sub-family	Gene name	Locus	Size (AA)	Best hit EST	Topology	Old name	Expression												Abs value
							L	R	B	F	F1	F2	F3	M	Bk	Rd			
	SIABCG65	<i>Solyc07g065780.1</i>	446	-	NBD													15.2	
	SIABCG66	<i>Solyc11g018680.1</i>	291	SGN-E717727	NBD													19.9	
	SIABCG67	<i>Solyc00g164680.1</i>	491	-	NBD													28.9	
	SIABCG68	<i>Solyc02g055530.2</i>	59	-	NBD													39.4	
	SIABCG69	<i>Solyc04g076170.1</i>	190	-	NBD													18.9	
	SIABCG70	<i>Solyc09g042280.1</i>	112	-	NBD													7.20	
ABCI	SIABCI1	<i>Solyc00g304030.1</i>	1,081	-	NBD													0.00	
	SIABCI2	<i>Solyc01g100850.2</i>	329	SGN-E1301393	NBD													52.8	
	SIABCI3	<i>Solyc02g068180.2</i>	275	SGN-E1307012	NBD													22.3	
	SIABCI4	<i>Solyc03g117810.2</i>	264	SGN-E1270799	NBD													105	
	SIABCI5	<i>Solyc04g056650.2</i>	351	SGN-E700042	NBD													24.1	
	SIABCI6	<i>Solyc06g048540.2</i>	313	SGN-E720007	NBD													130	
	SIABCI7	<i>Solyc06g068600.2</i>	186	-	NBD													116	
	SIABCI8	<i>Solyc09g066470.2</i>	287	SGN-E321321	NBD													79.0	
	SIABCI9	<i>Solyc11g069260.1</i>	261	SGN-E302237	NBD													19.4	
	SIABCI10	<i>Solyc12g010220.1</i>	230	SGN-E203090	NBD													8.15	

The best hit ESTs were found by blasting from SGN web server (<https://solgenomics.net/>). Pfam web server (<http://pfam.xfam.org/>) was used to identify the conserved domains (topology); NBD: nucleotide binding domain (ATP binding cassette domain); TMD: transmembrane domain. Gene expression profile data in various tomato organs and tissues was obtained from Tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi). The gene expression levels (low to high) are indicated by the light to deep red color shades. L: leaf; R: root; bud; F: flower; F1: 1cm fruit; F2: 2cm fruit; F3: 3cm fruit; M: mature green; Bk: breaker; Rd: 10 days after breaker; Abs value: RPKM value of maximum gene expression level in various tomato organs and tissues for each gene.

<https://doi.org/10.1371/journal.pone.0200854.t001>

another tomato genome database, TMCSv1.2.1 from TOAMTOMICS [23,24] (S3 Table). As a result, no new tomato ABC proteins were found in TMCSv1.2.1. However, corresponding genes of *SIABCA8*, *SIABCC22*, *SIABCC24* and *SIABCG68* identified in SL3.0 were not identified in TMCSv1.2.1 (S3 Table). The tomato eFP browser showed gene expression data for *SIABCA8*, *SIABCC22* and *SIABCG68* (Table 1), suggesting that these genes may be functional genes. On the other hand, the tomato eFP browser showed no gene expression for *SIABCC24* (Table 1), suggesting that *SIABCC24* may have been mispredicted. The SL3.0 tomato genome database suggests only one transcript for one locus, on the other hand, TMCSv1.2.1 suggests several splicing variants for one locus (S3 Table).

Wider research coverage on ABC transporters has caused emergence of several naming schemes. In most cases, they were named based on the mutant characteristics. This eventually resulted in assigning different names to the same subfamily or selected members with common characteristics [35]. To conform to plant and animal ABC communities, the Human Genome Organization (HUGO) nomenclature system [35] was adopted to designate all putatively ABC proteins into their diverse subfamilies (Fig 1). A unified ABC nomenclature proposed by Verrier et al. [35] was also used to assign ABCA-ABCG and ABCI to all the eight subfamilies (Table 1).

The 154 ABC proteins identified in the tomato genome were grouped into 9 ABCAs, 29 ABCBs, 26 ABCCs, 2 ABCDs, 2 ABCEs, 6 ABCFs, 70 ABCGs and 10 ABCIs (Table 1, Fig 1). The most abundant subfamily members were ABCB, ABCC and ABCG; while ABCD and ABCE were the least abundant. This characteristic is similar to the distribution of ABC

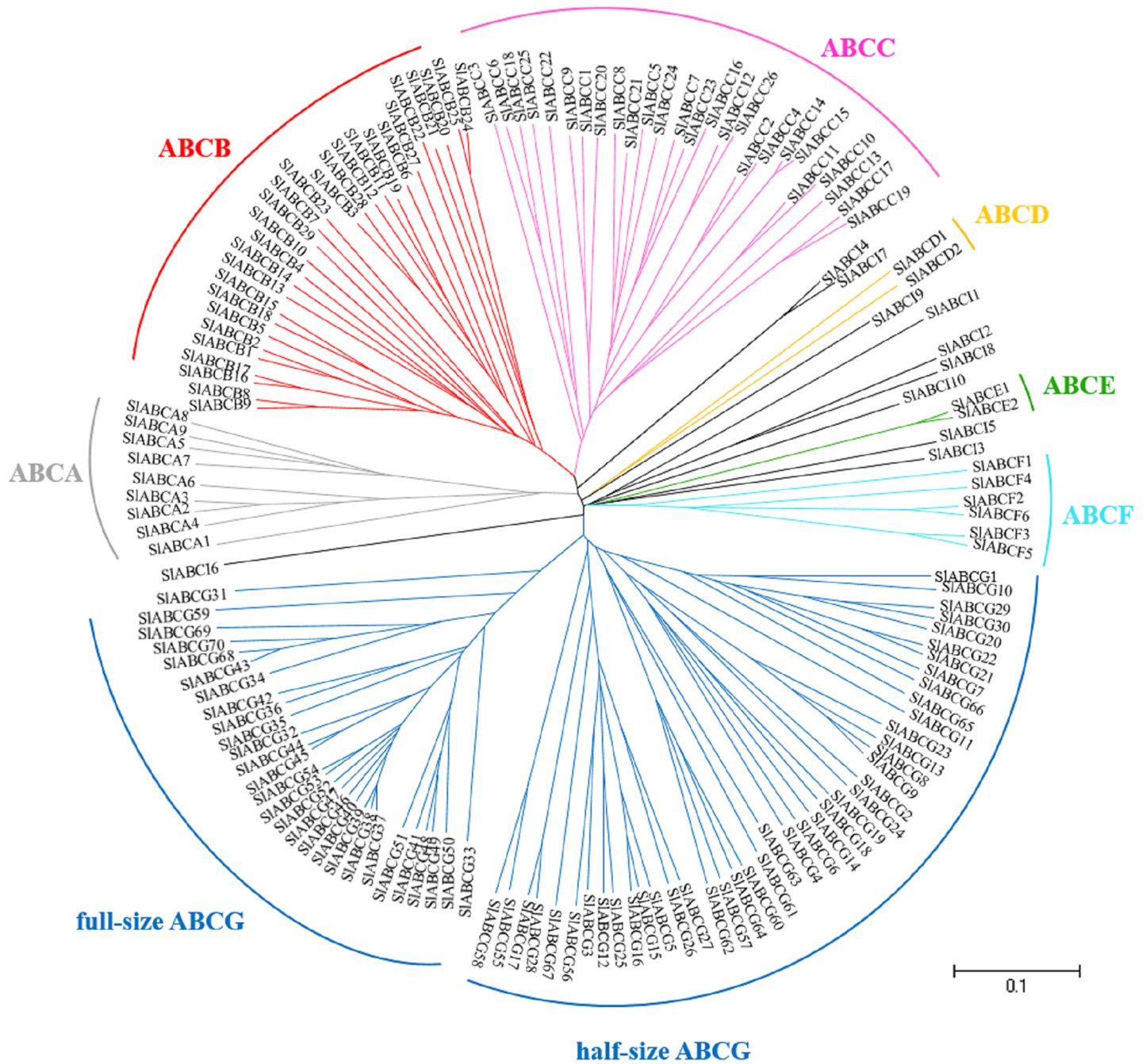


Fig 1. Phylogenetic tree of tomato ABC proteins. The 154 ABC proteins identified were subjected to phylogenetic analysis. Subfamily names (ABCA-I, except ABCB) correspond to the mammalian ABC transporter nomenclature. Tomato ABC proteins not clustered in ABCA-ABCG subfamilies are ABCIs. The scale indicated in the figure shows 10% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g001>

proteins in human [36] and other plants, such as Arabidopsis [15], rice [16], *L. japonica* [18] and *H. brasiliensis* [4]. At least one EST in the SGN database (<http://www.solgenomics.net/>) [21] was found for 78 genes. The reason for the absence of ESTs for the 69 genes could be that they are either expressed only under certain conditions or in specific cell types. Alternatively,

they could represent pseudogenes as suggested in genome-wide analysis of tomato aquaporins and sugar transporters [37,38].

A typical full-size of ABC protein has >1,200 amino acid residues [39]. The sizes of the 154 ABC proteins of tomato ranged from 50 to over 1,910 amino acid residues, although all of them possess at least one NBD as shown in Table 1. Some of the tomato ABC proteins with shorter sequences might be pseudogene or misannotation as suggested in the genome-wide analysis of tomato aquaporins and sugar transporters [37,38]. Among the 154 tomato ABC proteins, 47 members are lacking a TMD and are considered as soluble ABC proteins (Table 1). On the other hand, the other 107 members possess TMDs and are considered as ABC transporters.

One of the unique features of ABC proteins is their topological diversity. Structural orientation and conserved domains for each protein predicted by the Pfam web server is shown in Table 1. Fifty-four ABC proteins are full-size proteins possessing (TMD-NBD) \times 2. Among these members, 32 exhibit a forward, while 22 have a reverse topology orientations. Fifty-three ABC proteins were half-size having (TMD-NBD) \times 1 or (NBD-TMD) \times 1. Among the half-size ABC proteins, 18 exhibit a forward and 35 a reverse domain orientations. Forty-seven ABC proteins are considered as quarter-size ABC transporter proteins. *SlABCB19* and *SlABCC13* were uniquely characterized with NBD-TMD-NBD and TMD-NBD-TMD orientations, respectively. Similar topological patterns were reported in ABC proteins of rice [16], maize [17] and *L. japonica* [18]. Such characteristics might have resulted from gene duplication or evolved to render specific physiological functions [40].

The tomato ABC protein subfamilies

ABCA subfamily. The plant ABCA subfamily is made up of one full-size ABCA and several half-size ABCAs. In Arabidopsis, AtABCA1, also known as ABC one homologue (AOH), is the only full-size ABCA protein and is the largest ABC protein, consisting of 1,882 amino acid residues [15,16]. The remaining are half-size ABCAs are also called ABC two homologues (ATH). In tomato genome, 9 members of the ABCA subfamily were found (Table 1, Fig 2). *SlABCA1* was the only full-size ABCA and the largest ABC protein identified, consisting of 1,910 amino acids residues (Table 1). On the other hand, 6 half-size and 2 quarter-size ABCAs were found in tomato genome. A major feature of the ABCA subfamily is the presence of one AOH full-size ABCA in dicots, including tomato (Table 1), Arabidopsis [15], *L. japonicas* [18] and grape [19], that so far has not been identified in monocots, such as rice [16] and maize [17]. This suggests that the function of this full-size ABCA is specific to dicots.

The functions of ABCAs in plants are currently almost unknown, although mammalian ABCAs have been shown to be involved in numerous functions, such as lipid metabolism, cholesterol homeostasis, intracellular trafficking, pulmonary surfactant secretion and retinal transport [41]. AtABCA1 was reported to be related in pollen germination, seed germination and seed maturation [18,19]. Transcriptome analysis in Arabidopsis roots has revealed that *AtATH14* and *AtATH15* expressions are responsive to salt stress [42]. Among the 9 *SlABCAs*, ESTs of 5 members were available. The gene expression profiles from the eFP Browser revealed that *SlABCA1* and *SlABCA2* are preferentially expressed in the root (Table 1) and they might be involved in secretion activity of roots. *SlABCA4-7* are expressed specifically in the flower, suggesting a specific functions in floral organs (Table 1).

ABCB subfamily. The ABCB subfamily is the second largest subfamily. Full-size ABCBs are known as multidrug resistance protein (MDR) or P-glycoprotein (PGP) and the half-size ABCBs are characterized with names such as transporter associated with antigen processing (TAP), ABC transporter of mitochondria (ATM) and lipid A-like exporter putative (LLP) [35].

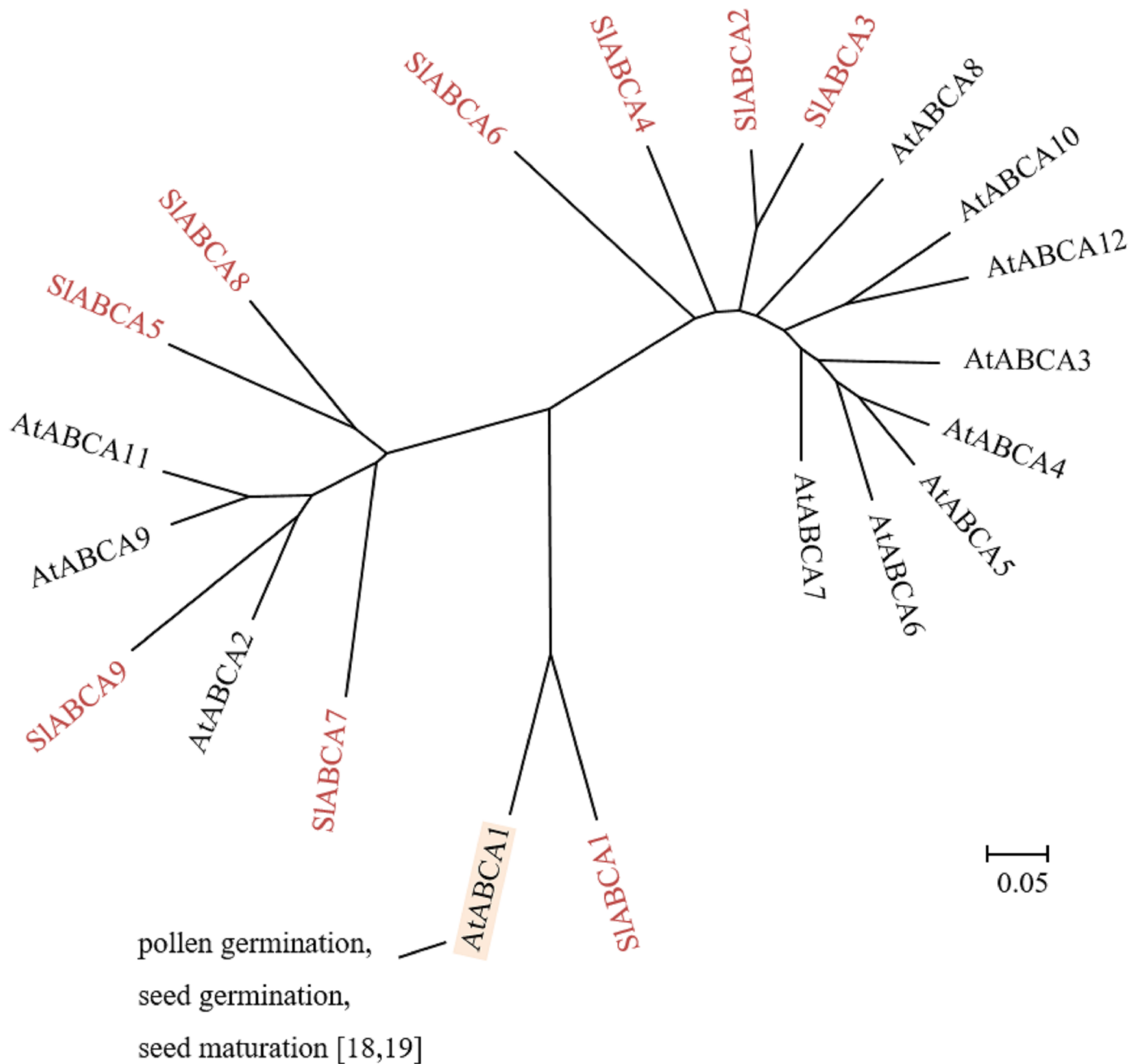


Fig 2. Phylogenetic tree of plant ABCA subfamily. ABCAs of tomato and Arabidopsis were subjected to phylogenetic analysis. Tomato ABCAs are shown in red. Physiological functions and references are indicated. The scale indicated in the figure shows 5% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g002>

In the tomato genome, 29 members of this ABCB subfamily were identified and this comprises 18 full-size, 8 half-size and 3 quarter-size (Table 1) while in Arabidopsis, 22 full-size proteins, 6 half-size proteins and no quarter-size are identified. Surprisingly, according to the database, SIABCB18 contains 5 domains, i.e. TMD-TMD-NBD-TMD-NBD, and SIABCB19 contains 3 domains, i.e. NBD-TMD-NBD. These unique topological arrangements, i.e. additional TMDs or NBDs in their forward orientations may be caused by a prediction error for the CDS or indicate that these sequences are pseudogenes (Table 1).

All the characterized full-size ABCBs in *Arabidopsis* are localized to the plasma membrane [43,44], whereas the half-size ABCBs, ATMs (AtABCB23–25) have been reported to reside in mitochondria [45,46] while TAPs (AtABCB26 and AtABCB27) have been detected in the chloroplast [47] and vacuolar membrane [8,48]. In humans, ABCBs are associated with multi-drug resistance [36], lipid transport [49], iron and peptide transports [50]. Plant ABCBs are associated with several physiological functions as shown in Fig 3. For instance, AtABCB1 [51], AtABCB4 [52], AtABCB14, AtABCB15 [53], AtABCB19 [54] and AtABCB21 [55] are implicated in auxin transport in *Arabidopsis*. AtACBB14 was also reported to be associated with regulation of stomatal opening and closing [44]. AtABCB23, AtABCB24 and AtABCB25 modulate Fe-S cluster biogenesis [56]. AtABCB25 is involved in molybdenum cofactor biosynthesis and heavy metal tolerance, probably through their function as glutathione disulfide (GSSG) transporters [57]. AtABCB27 and its homologue in barley, HvMDR2 are responsible for Al and Fe sequestration respectively [58,59]. In *Coptis japonica*, CjMDR1 transports berberine [60]. In wheat, TaMDR1 modulates aluminum toxicity responses and cadmium homeostasis [61]. In *Chlamydomonas reinhardtii*, CrCds1 mediates tolerance to cadmium [61,62].

In tomato, only 10 ESTs out of 29 the SIABCBs were available (Table 1, Fig 3). Based on the eFP Browser gene expression data, SIABCB7, SIABCB13, SIABCB14, SIABCB18, SIABCB20, SIABCB21, SIABCB24, SIABCB25 and SIABCB29 are ubiquitously expressed in all organs and tissues (Table 1), suggesting their responsibilities for basic cellular maintenance. Most of SIABCBs are highly expressed in the root. This may suggest an involvement of these SIABCBs in ion and heavy metal transports in roots.

ABCC subfamily. ABCCs are also called multidrug resistance-associated proteins (MRP) due to their function in transporting glutathione- and glucuronide-conjugates in drug-resistant animal cancer cells [35]. In plants, full-size ABCCs were earlier characterized and later half-size ABCCs were found in *Arabidopsis* and rice genomes and characterized [4,17]. In plants, most ABCCs are characterized as vacuolar localized proteins and few have been reported to reside on the plasma membrane [17]. Maize ZmMRP3 and grape VvABCC1 are involved in anthocyanin accumulation in vacuoles [6,7]. *Arabidopsis* AtABCC1–4 and wheat TaMRP1 are involved in transport of glutathione-conjugates [63]. *Arabidopsis* AtABCC5 [64], maize ZmMRP4 [65] and rice OsABCC13 [66] are implicated in phytate transport [67]. AtABCC2 and AtABCC3 are involved in chlorophyll catabolite transport [63]. AtABCC1 and AtABCC4 are implicated in folate transport [63]. AtABCC4 and AtABCC5 are functionally related to stomatal regulation [63]. AtABCC3, AtABCC6 and AtABCC7 confer heavy metal resistance [68,69].

In the tomato genome, 26 members of the ABCC subfamily were found and this comprises 12 full-size, 6 half-size and 8 quarter-size ABCCs. SIABCC13 shows a unique protein structure, i.e. TMD–NBD–TMD (Table 1, Fig 4), however as for the non-typical ABCBs this might reflect a prediction error for the CDS or the presence of a pseudogene. SIABCC18 shows reverse orientation (NBD–TMD), which is different from other SIABCCs (TMD–NBD). ESTs for 11 ABCCs were available (Table 1). The gene expression profile of the tomato eFP Browser shows that SIABCC1, SIABCC7, SIABCC10, SIABCC11, SIABCC13, SIABCC19, SIABCC20 and SIABCC21 are preferentially expressed in the later stages of fruit development (Table 1). These SIABCCs might play important roles in fruit ripening, such as chlorophyll degradation and secondary metabolite accumulation in the vacuole.

ABCD subfamily. ABCDs are also known as peroxisomal membrane proteins (PMPs) and are localized in the peroxisomal membrane [70,71]. In humans, they are exclusively known to be half-size proteins with TMD–NBD orientation, whereas, in plants, both half- and full-size ABC proteins exist [15]. AtABCD1 is implicated in benzoic (BA) synthesis [72], transport of 12-oxophytodienoic acid (OPDA) [73] and jasmonic acids (JA) [74]. The *AtABCD1*

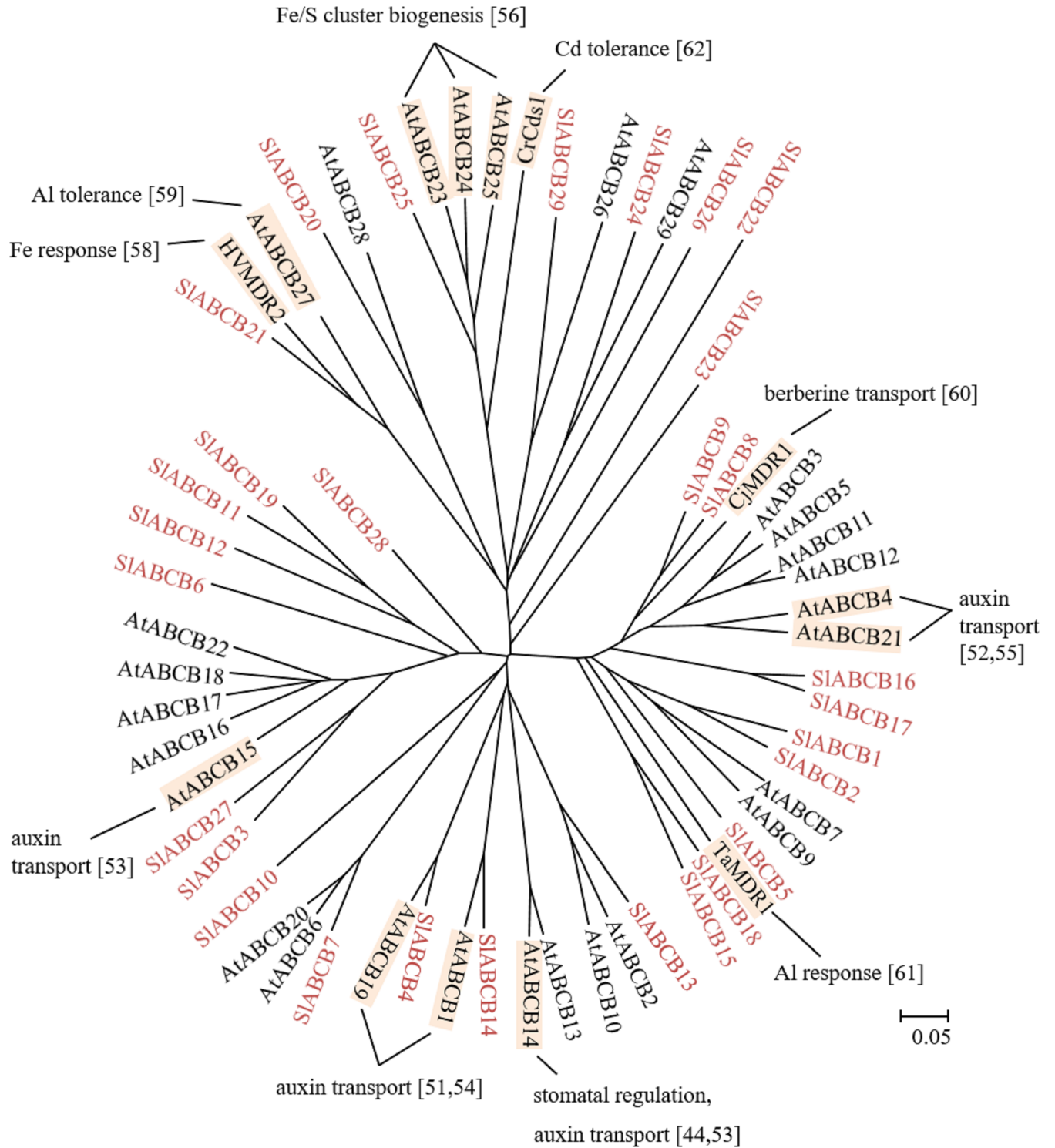


Fig 3. Phylogenetic tree of plant ABCB subfamily. ABCBs of tomato, Arabidopsis, barley (HvMDR2: BAC53613), wheat (TaMDR1: BAB85651), *Coptis japonica* (CjMDR1: BAB62040) and *Chlamydomonas reinhardtii* (CrCds1: AAQ19846) were subjected to phylogenetic analysis. Tomato ABCBs are shown in red. Physiological functions and references are indicated. The scale indicated in the figure shows 5% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g003>

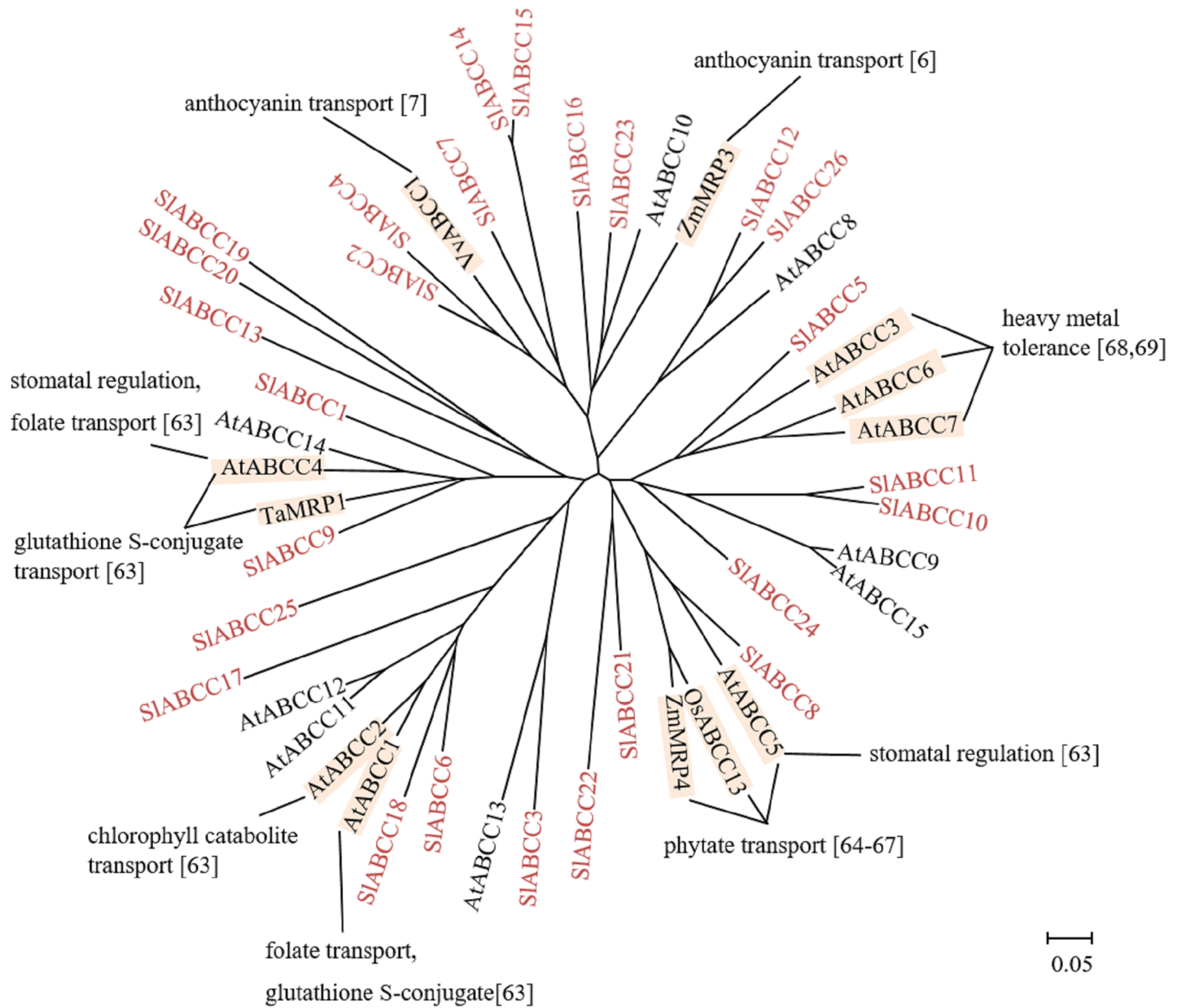


Fig 4. Phylogenetic tree of plant ABCC subfamily. ABCCs of tomato, Arabidopsis, rice (*OsABCC13*: *Os03g0142800*), maize (*ZmMRP3*: AAT37905, *ZmMRP4*: ABS81429), wheat (*TaMRP1*: AAL47686) and grape (*VvABCC1*: AGC23330) were subjected to phylogenetic analysis. Tomato ABCCs are shown in red. Physiological functions and references are indicated. The scale indicated in the figure shows 5% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g004>

mutant is impaired in seed germination [75] and fertility [76]. The tomato genome contains one full-size and one half size ABCDs were found (Table 1, Fig 5). The gene expression profile of the tomato eFP Browser shows constitutive gene expression of both *SIABCCs* (Table 1). It is likely that these transporters exhibit similar functions as their Arabidopsis counterparts and that they are involved in peroxisomal import of long chain fatty acids.

ABCE subfamily. ABCEs, also called RNase L inhibitor (RLI), possess an N-terminal Fe-S domain, which interacts with nucleic acids [30]. All ABCE subfamily members are soluble ABC proteins harboring two conserved NBDs (NBD-NBD) [17]. In humans, only one ABCE

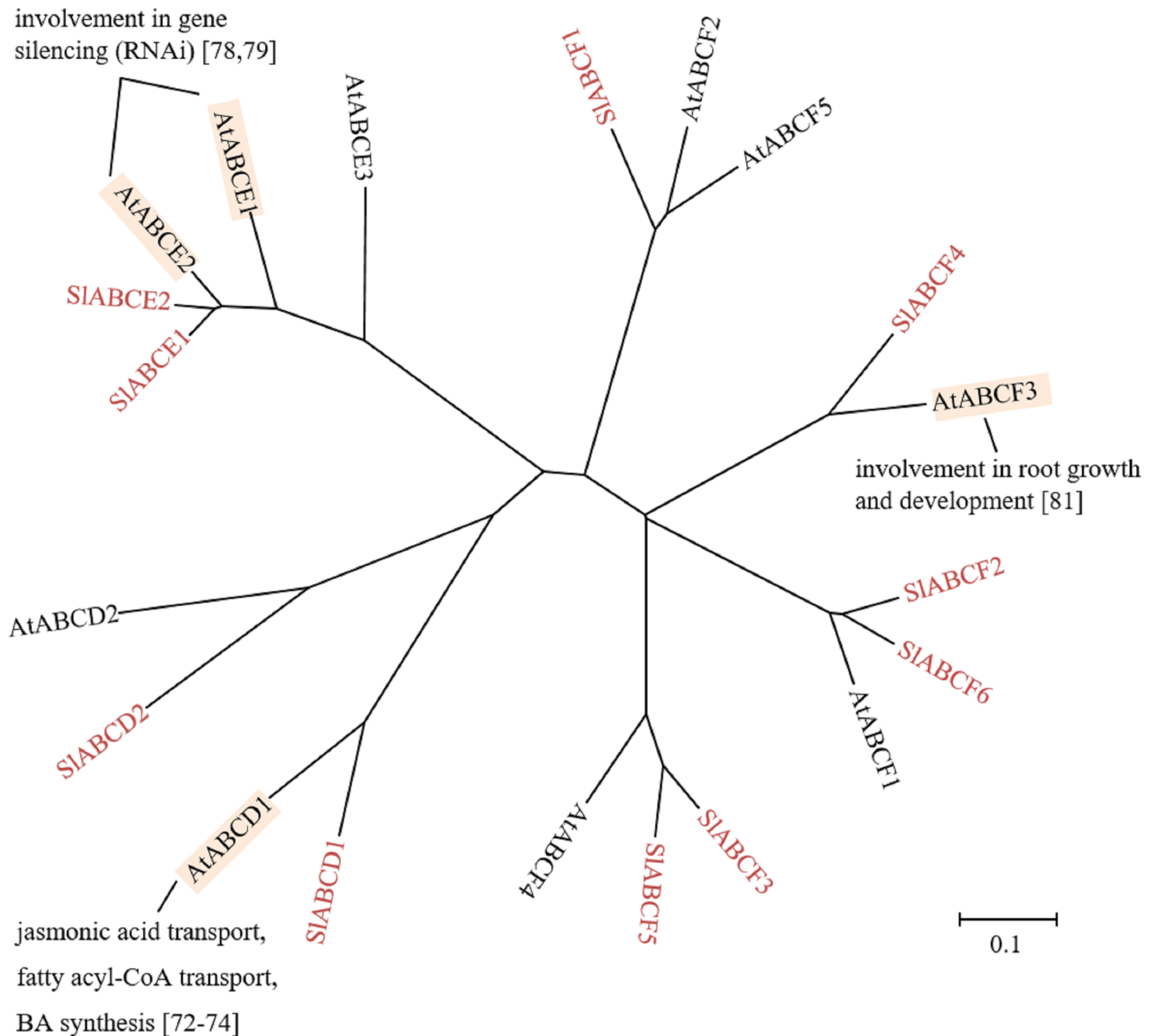


Fig 5. Phylogenetic tree of plant ABCD, ABCE and ABCF subfamilies. ABCDs, ABCEs and ABCFs of tomato and Arabidopsis were subjected to phylogenetic analysis. Tomato ABC proteins are shown in red. Physiological functions and references are indicated. The scale indicated in the figure shows 10% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g005>

exists and it is involved in ribosome biogenesis and control of translation [77]. There are 3 ABCEs present in Arabidopsis and two each in rice [16], maize [17], grape [19], *L. japonicas* [18], *H. brasiliensis* [4] and also in tomato (Table 1, Fig 5). In Arabidopsis, AtABCE1 and ABCE2 are involved in RNA interference (RNAi) regulation [78,79]. Among the two tomato *SlABCEs*, only one EST of *SlABCE1* was available (Table 1). The tomato eFP Browser revealed that both *SlABCE1* and *SlABCE2* are expressed constitutively in all organs and tissues (Table 1)

and may play roles in ribosome biogenesis, control of translation and gene silencing regulation.

ABCF subfamily. ABCFs are also called general control non-repressible homologs (GCN). The ABCF subfamily is similar to the ABCE subfamily [17], because ABCFs are also soluble ABC proteins containing two fused NBDs (NBD-NBD). In yeast and humans, ABCFs are involved in gene expression regulation [16,80]. In Arabidopsis, 5 ABCFs are present and AtABCF3 is implicated in root growth [81]. In tomato, 6 ABCFs were identified and ESTs were available for 5 ABCFs (Table 1, Fig 5). The Tomato eFP Browser showed constitutive expressions for all 6 *SlABCFs* (Table 1).

ABCG subfamily. The ABCG subfamily is the largest subfamily in plants while only 5 ABCGs are present in humans [17]. The ABCG subfamily is made up of full-size and half-size ABC proteins, also called pleiotropic drug resistance (PDR) or white-brown complex (WBC), respectively [35]. All full-size and half-size ABCGs have two, respectively one NBD-TMD, and function as ABC transporters. In the tomato genome, 70 ABCGs were found, which are made up of 22 full-size, 32 half-size and 16 quarter-size ABC proteins (Table 1). This number is larger than the 44 ABCGs reported for Arabidopsis [15]. In humans, ABCGs function as transporters of cholesterol, urate, haem, and other pharmaceutical compounds [82]. On the other hand, in plants, ABCGs have been reported to transport various phytohormones, including abscisic acid (ABA), cytokinin, strigolactone and auxin derivatives [10].

One of the most widely studied ABC protein subfamily in plants are the full-size ABCGs, also called PDRs. A detailed review on plant full-size ABCGs is available [83,84] and a highlight on their functions is shown in Fig 6. The subcellular localization of full-size ABCGs is the plasma membrane [84]. Full-size ABCGs of Arabidopsis AtABCG32 [85], rice OsABCG31 [86], barley HvABCG31 [86] are involved in cuticle formation. The *N. plumbaginifolia* NpPDR1 [87] and duckweed SpTUR2 are known to participate in sclareol transport [88].

Half-size ABCGs are also called WBCs, have been reported to be localized in the plasma membrane, mitochondrial membrane, chloroplast membrane and cytoplasm [17]. The physiological roles of half-size ABCGs are summarized in Fig 7. In Arabidopsis, half-size ABCGs, i.e. AtABCG11-13 are implicated in cuticle formation [89–91]. On the other hand, AtABCG19 confers kanamycin resistance [9]. AtABCG25 has been reported to act as an ABA exporter [92] and AtABCG26 is involved in pollen development [93]. In cotton, GhWBC1 is involved in cotton yarn expansion [94].

The tomato eFP browser shows specific expressions of *SlABCG12*, *SlABCG16*, *SlABCG31*, *SlABCG32*, *SlABCG44*, *SlABCG45*, *SlABCG51*, *SlABCG52*, *SlABCG55* and *SlABCG58* (Table 1), suggesting their importance in root. *SlABCG25*, *SlABCG27*, *SlABCG29*, *SlABCG30*, *SlABCG43*, *SlABCG65*, *SlABCG68* and *SlABCG70* are expressed specifically in bud. Interestingly, only *SlABCG59*, which encodes a quarter-size ABCG, shows specific expression in mature fruit, although other *SlABCGs* are also expressed in fruits. Although we cannot guess the function of *SlABCG59*, it may play an important roles in tomato fruit maturation.

ABCI subfamily. ABCIs are also called non-intrinsic ABC proteins (NAPs). ABCIs are soluble ABC proteins possessing a single ATP binding domain [35]. In Arabidopsis, AtABCI1 and AtABCI2 are reported to be involved in cytochrome c maturation (CCM) [95]. AtABCI6-8 are implicated in biosynthesis of Fe/S cluster [96,97]. AtABCI13-15 are responsible for plastid lipid formation [97]. On the other hand, AtABCI16 and AtABCI17 confer tolerance to aluminum [8]. In the tomato genome, 10 *SlABCI*s have been identified and ESTs for 8 *SlABCI*s were available (Table 1, Fig 8). The gene expression profiles from the tomato eFP Browser showed that *SlABCI4*, *SlABCI6* and *SlABCI8* are constitutively expressed in roots and floral organs, respectively, and *SlABCI5*, *SlABCI6*, *SlABCI9* and *SlABCI10* in developing fruits (Table 1), suggesting their specific functions in these organs and tissues.

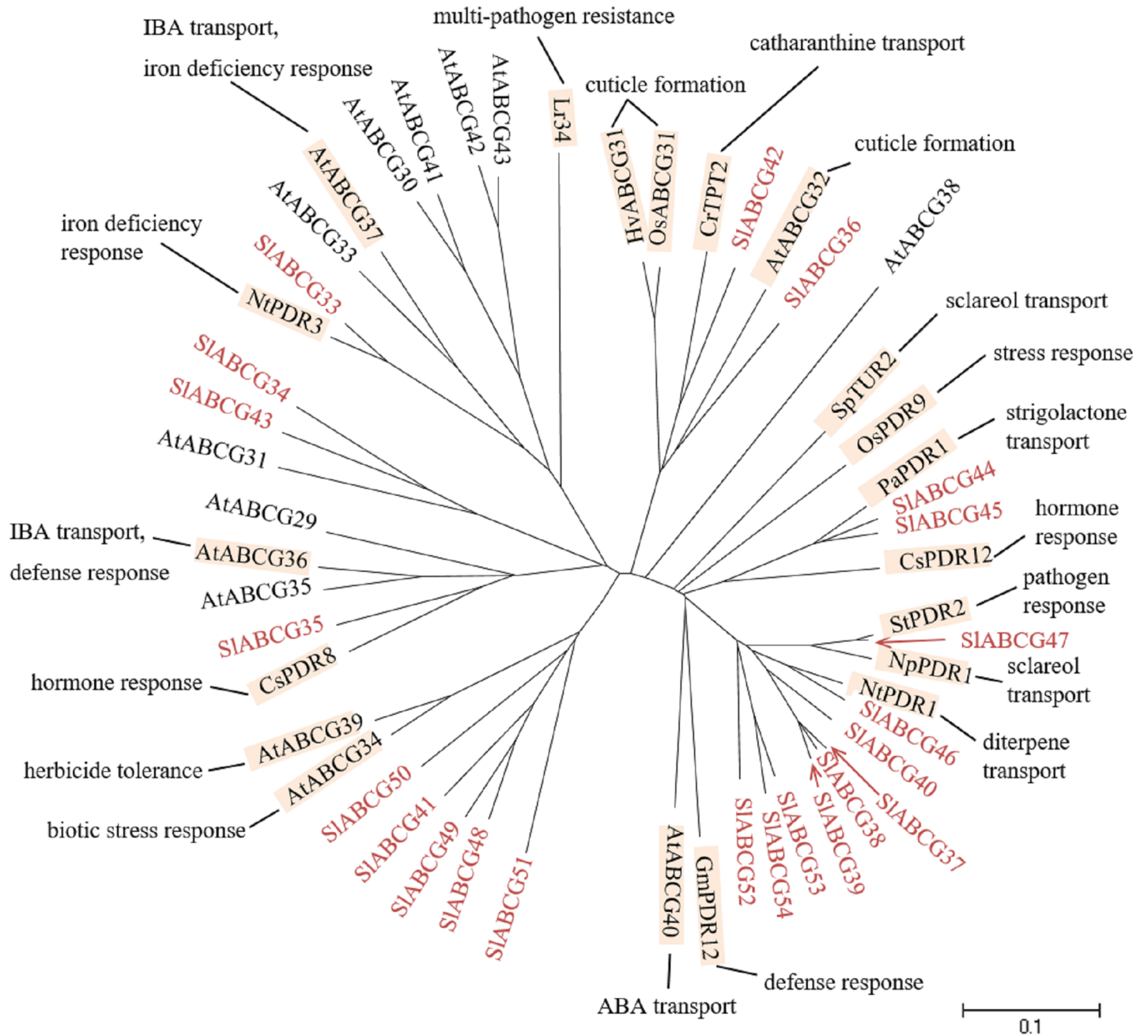


Fig 6. Phylogenetic tree of plant full-size ABCGs. ABCGs of tomato, Arabidopsis, rice (OsABCG31: *Os01g0177900*, OsPDR9: *Os01g0609300*), wheat (Lr34: ACN41354), barley (HvABCG31: NP_001237697), soybean (GmPDR12: NP_001237697), cucumber (CsPDR8: ACU82514, CsPDR12: ACU82515), *Nicotiana plumbaginifolia* (NpPDR1: Q949G3, NpPDR2: CAH40786), *N. tabacum* (NtPDR1: AGN95757, NtPDR3: CAH39853), petunia (PaPDR1: AFA43816), potato (StPDR2: AEB65936), periwinkle (CrTPT2: KC511771) and duckweed (SpTUR2: CAA94437) were subjected to phylogenetic. Tomato ABCGs are shown in red. Physiological functions and references are indicated. Details on the functions are reviewed in [83,84]. The scale indicated in the figure shows 10% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g006>

Gene expression analysis

We chose SIABCB4, SIABCC11, SIABCG7, SIABCG8, SIABCG9, SIABCG12, SIABCG13, SIABCG17, SIABCG22, SIABCG28 and SIABCG36 for further gene expression analysis by RT-

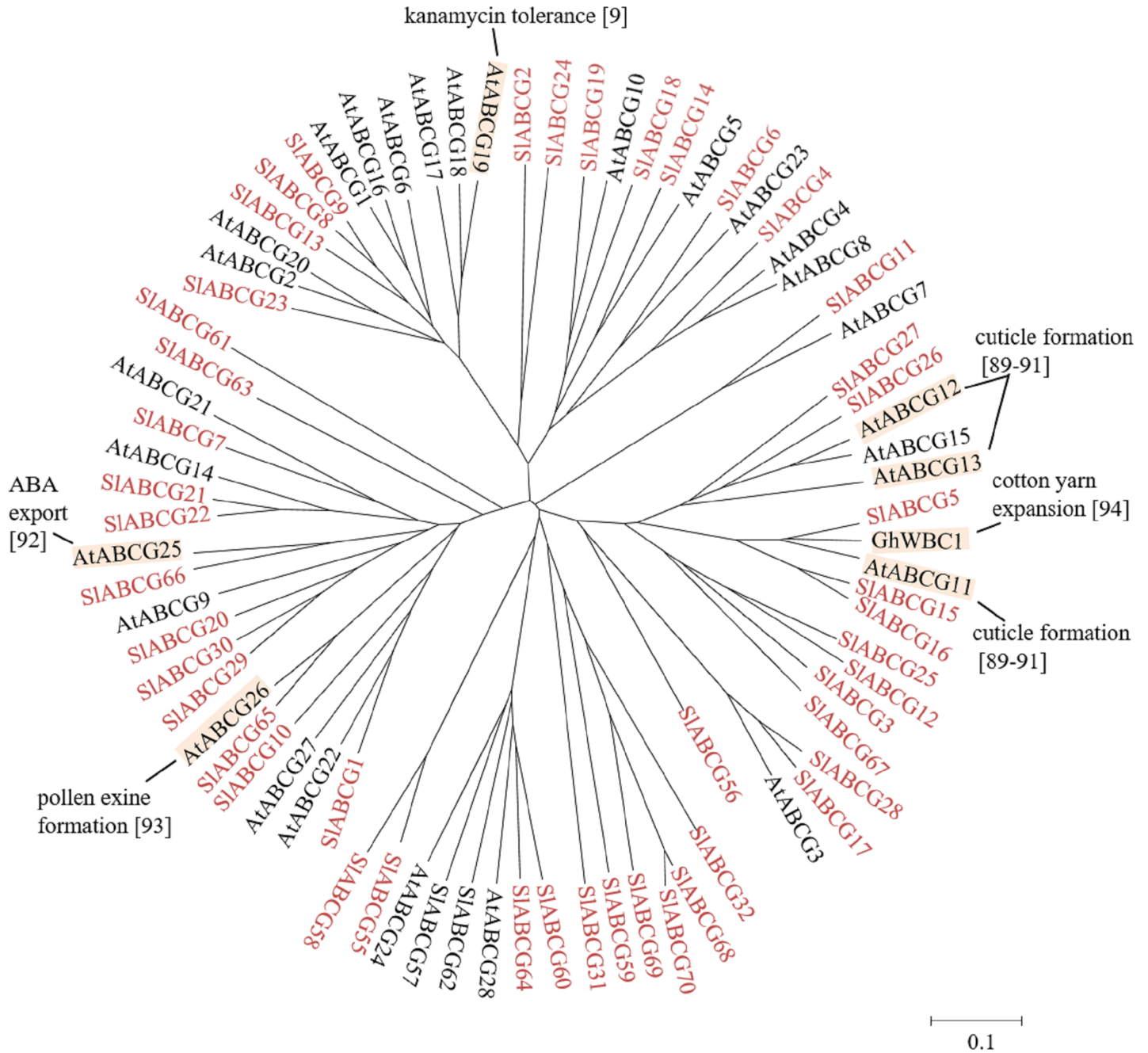


Fig 7. Phylogenetic tree of plant half-size ABCGs. ABCGs of tomato, Arabidopsis and cotton (GhWBC1: AAP80385) were subjected to phylogenetic analysis. Tomato ABCGs are shown in red. Physiological functions and references are indicated. The scale indicated in the figure shows 10% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g007>

sqPCR (Fig 9). These genes were chosen because their full length cDNA sequences were available in TOMATOMICS database (<http://plantomics.mind.meiji.ac.jp/tomatomics/>). Therefore, we requested for their full length cDNA clones from National Bioresource Project (NBRP)-Tomato (<http://tomato.nbrp.jp/indexEn.html>) to sequence and then performed RT-sqPCR to identify their expression patterns.

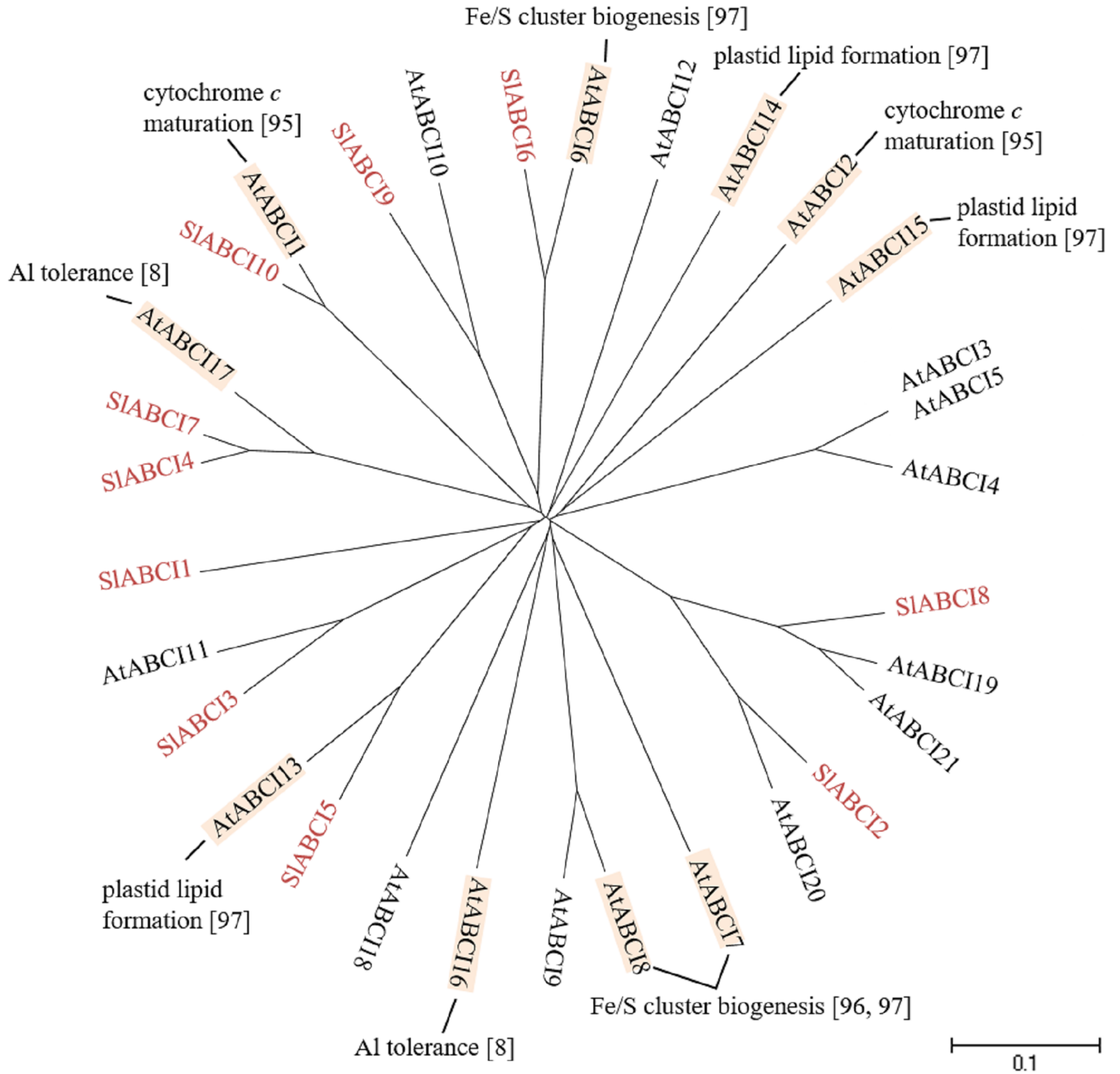


Fig 8. Phylogenetic tree of plant ABCI subfamily. ABCIs of tomato and Arabidopsis were subjected to phylogenetic analysis. Tomato ABCIs are shown in red. Physiological functions and references are indicated. The scale indicated in the figure shows 10% divergence between protein sequences.

<https://doi.org/10.1371/journal.pone.0200854.g008>

Gene expression was detected in various organs of 'MicroTom', i.e. leaf, stem, root, flower and developing fruits. In addition, to obtain a detailed gene expression profile in fruits, gene expressions in fruit peel and flesh at 10 DAP, breaker and red stages were investigated.

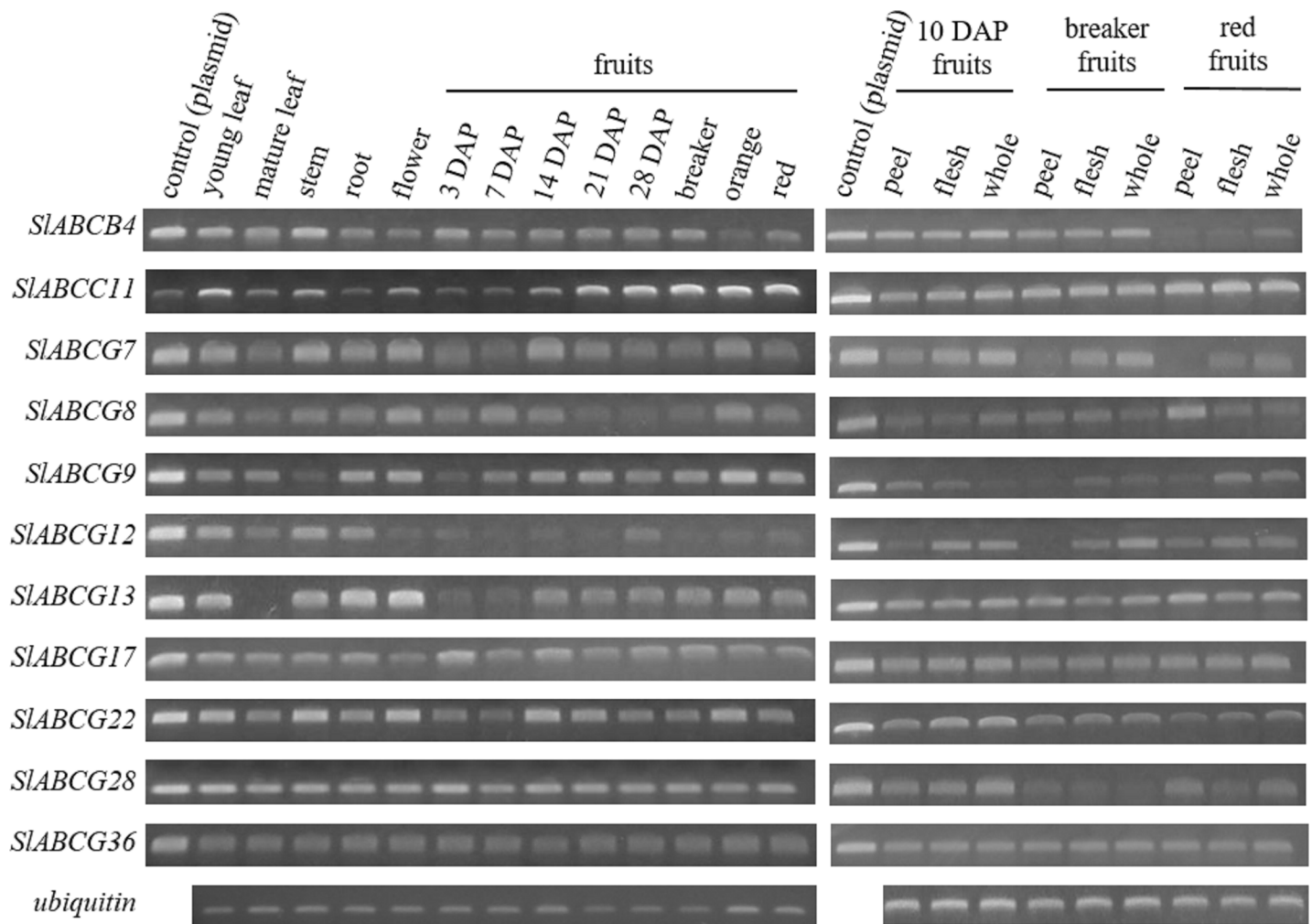


Fig 9. Gene expression analysis of selected ABC transporters in various tomato organs and tissues. RT-sqPCR analysis for selected tomato ABC transporters was performed using RNA extracted from the indicated organ or tissue and gene-specific primers (amplicons ~ 200 bp). Respective cDNA-containing plasmid was used as control. The ubiquitin gene was used as a constitutively expressed control gene. DAP: days after pollination.

<https://doi.org/10.1371/journal.pone.0200854.g009>

Although most *SlABCs* were ubiquitous expressed, some *SlABCs* exhibited a characteristic gene expression patterns (Fig 9).

SlABCB4 showed ubiquitous expression, but its transcript level was lower in mature fruits (Fig 9). The closest orthologue of *SlABCB4* in Arabidopsis is *AtACB19*, and has been reported to transports auxin [98]. This suggests that *SlABCB4* might be responsible for auxin transport in various organs of tomato. *SlABCC11* expression was high in mature leaf and fruits after 21 DAP (Fig 9). Although the function of *SlABCC11* is unclear because no close orthologue of Arabidopsis exists (Fig 3), it may play important roles in the later part of tomato fruit development.

Functions of half-size *SlABCGs*, *SlABCG7*, *SlABCG8*, *SlABCG9*, *SlABCG12*, *SlABCG13*, *SlABCG17*, *SlABCG22* and *SlABCG28* are unclear, because no characterized orthologue exists (Fig 7). *SlABCG7*, *SlABCG8*, *SlABCG9*, *SlABCG12*, *SlABCG13*, *SlABCG17*, *SlABCG22* and *SlABCG28* showed different expression patterns and *SlABCG9*, *SlABCG13*, *SlABCG17*, *SlABCG22* and *SlABCG28* showed relatively higher expression levels in fruits (Fig 9), suggesting that they may play some their roles in fruit development and/or ripening.

SLABCG36, which encode a full-size *SLABCG*, showed ubiquitous expression in all organs (Fig 9). *SLABCG36* is likely to transport metabolites involved in cuticle formation, because its closest orthologue of Arabidopsis, *AtABCG32* is responsible for cuticle formation (Fig 6) [85]. Therefore we expected high *SLABCG36* expression in fruit peel. However, the differences in *SLABCG36* expressions between in fruit peel and flesh were not pronounced, although it was slightly higher in the peel than in flesh of red fruit (Fig 9).

Conclusion

This study revealed the presence of 154 putative ABC proteins in the tomato genome. Based on the phylogenetic analysis, the ABC proteins were grouped into their respective subfamilies, ABCA through to ABCI, except ABCH. Members of ABCG, ABCB and ABCC subfamilies were the most abundant, while ABCD and ABCE subfamilies were less abundant. Among the 154 tomato ABC proteins, 47 members are soluble ABC proteins, while 107 members encode for ABC transporters with TMDs. As far as we know, this study is the only genome-wide analysis of ABC proteins in the Solanaceae species. In this study, we provided the fundamental and exhaustive information about tomato ABC proteins, i.e. the list of all ABC proteins in tomato with their locus numbers (gene IDs), protein topology, best hit ESTs, gene expression data (Table 1) and phylogenetic trees of subfamily members and orthologues in other plants, showing the reported physiological functions (Figs 2–8). This information is indispensable for further studies of ABC proteins not only in tomato but also in other Solanaceae species. We hope this study will be useful to many researchers studying plant ABC proteins.

Supporting information

S1 Table. Primers and PCR conditions for RT-sqPCR. The forward and reverse primers, PCR condition and number of PCR cycles for each ABC transporter or control gene (*ubiquitin*) are shown.
(DOCX)

S2 Table. Comparison of non-overlapping tomato ABC proteins in this study and in Andolfo et al. 2015 and presence of nucleotide binding domain (NBD). The presence of nucleotide binding domain (NBD) was confirmed using Pfam web server (<http://pfam.xfam.org/>).
(XLSX)

S3 Table. Comparison of genes putatively encoding ABC proteins in two tomato genome databases, SL3.0 and ITAG3.10 from Sol Genomics Network and TMCSv1.2.1 from TOMATOMICS. Genes putatively encoding ABC proteins in TMCSv1.2.1 from TOMATOMICS (<http://plantomics.mind.meiji.ac.jp/tomatomics/download.php>) were obtained by blasting using the protein sequences (Table 1) from SL3.0 and ITAG3.10 of Sol Genomics Network (https://solgenomics.net/organism/Solanum_lycopersicum/genome). Identical genes between two different tomato genome databases and splicing variants were confirmed by comparing their positions in chromosome.
(XLSX)

Acknowledgments

We thank the National Bioresource Project (NBRP)-Tomato for providing cDNA clones. This work was supported partially by the Programme for Promotion of Basic and Applied Researchers for Innovations from Bio-oriented Industry from the Bio-oriented Technology

Research Advancement Institution (BRAIN), the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry from the Ministry of Agriculture, Forestry and Fisheries (MAFF), the Cross-ministerial Strategic Innovation Promotion Program (SIP) from the Cabinet Office, and the Grant-in-Aids for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS).

Author Contributions

Conceptualization: Katsuhiko Shiratake.

Funding acquisition: Katsuhiko Shiratake.

Investigation: Peter Amoako Ofori, Ayaka Mizuno, Mami Suzuki, Katsuhiko Shiratake.

Methodology: Stefan Reuscher, Shungo Otagaki, Shogo Matsumoto, Katsuhiko Shiratake.

Project administration: Katsuhiko Shiratake.

Resources: Koh Aoki, Daisuke Shibata.

Supervision: Enrico Martinoia, Katsuhiko Shiratake.

Writing – original draft: Peter Amoako Ofori.

Writing – review & editing: Katsuhiko Shiratake.

References

1. Rees DC, Johnson E, Lewinson O. ABC transporters: the power to change. *Nat Rev Mol Cell Biol.* 2009; 10:218–227. <https://doi.org/10.1038/nrm2646> PMID: 19234479
2. Vasilio V, Vasilio K, Nebert DW. Human ATP-binding cassette (ABC) transporter family. *Hum Genomics.* 2009; 3:281–290. <https://doi.org/10.1186/1479-7364-3-3-281> PMID: 19403462
3. Kretschmar T, Burla B, Lee Y, Martinoia E, Nagy R. Functions of ABC transporters in plants. *Essays Biochem.* 2011; 50:145–160. <https://doi.org/10.1042/bse0500145> PMID: 21967056
4. Zhiyi N, Guijuan K, Yu L, Longjun D, Rizhong Z. Whole-transcriptome survey of the putative ATP-binding cassette (ABC) transporter family genes in the latex producing laticifers of *Hevea brasiliensis*. *PLoS One.* 2015; 10:e0116857. PMID: PMC4304824 <https://doi.org/10.1371/journal.pone.0116857> PMID: 25615936
5. Lefevre F, Bajot A, Boutry M. Plant ABC transporters: time for biochemistry? *Biochem Soc Trans.* 2015; 43:931–936. <https://doi.org/10.1042/BST20150108> PMID: 26517906
6. Goodman CD, Casati P, Walbot V. A multidrug resistance-associated protein involved in anthocyanin transport in *Zea mays*. *Plant Cell.* 2004; 16:1812–26. <https://doi.org/10.1105/tpc.022574> PMID: 15208386
7. Francisco RM, Regalado A, Ageorges A, Burla BJ, Bassin B, Eisenach C, et al. ABCC1, an ATP binding cassette protein from grape berry, transports anthocyanidin 3-o-glucosides. *Plant Cell.* 2013; 25:1840–1854. PMID: PMC3694709 <https://doi.org/10.1105/tpc.112.102152> PMID: 23723325
8. Kang J, Park J, Choi H, Burla B, Kretschmar T, Lee Y, et al. Plant ABC transporters. *The Arabidopsis Book.* American Society of Plant Biologists. 2011; 9:e0153. <https://doi.org/10.1199/tab.0153> PMID: 22303277
9. Mentewab A, Stewart CN. Overexpression of an *Arabidopsis thaliana* ABC transporter confers kanamycin resistance to transgenic plants. *Nat Biotechnol.* 2005; 23:1177–1180. <https://doi.org/10.1038/nbt1134> PMID: 16116418
10. Borghi L, Kang J, Ko D, Lee Y, Martinoia E. The role of ABCG-type ABC transporters in phytohormone transport. *Biochem Soc Trans.* 2015; 43:924–930. PMID: PMC4613532 <https://doi.org/10.1042/BST20150106> PMID: 26517905
11. Geisler M, Aryal B, Di Donato M, Hao P. A critical view on ABC transporters and their interacting partners in auxin transport. *Plant Cell Physiol.* 2017; 58:1601–1604. <https://doi.org/10.1093/pcp/pcx104> PMID: 29016918
12. Theodoulou FL. Plant ABC transporters. *Biochim Biophys Acta.* 2000; 79–103. <https://doi.org/10.1199/tab.0153> PMID: 22303277

13. Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat Rev Genet.* 2015; 16:45–56. PMID: PMC4364438 <https://doi.org/10.1038/nrg3849> PMID: 25404111
14. Westlake CJ, Cole SPC, Deeley RG. Role of the NH₂-terminal membrane spanning domain of multidrug resistance protein 1/ABCC1 in protein processing and trafficking. *Mol Biol Cell.* 2005; 16:2483–92. <https://doi.org/10.1091/mbc.E04-12-1113> PMID: 15772158
15. Sánchez-Fernández R, Davies TG, Coleman JO, Rea PA. The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory. *J Biol Chem.* 2001; 276:30231–30244. <https://doi.org/10.1074/jbc.M103104200> PMID: 11346655
16. Garcia O, Bouige P, Forestier C, Dassa E. Inventory and comparative analysis of rice and Arabidopsis ATP-binding cassette (ABC) systems. *J Mol Biol.* 2004; 343:249–265. <https://doi.org/10.1016/j.jmb.2004.07.093> PMID: 15381434
17. Pang K, Li Y, Liu M, Meng Z, Yu Y. Inventory and general analysis of the ATP-binding cassette (ABC) gene superfamily in maize (*Zea mays* L.). *Gene.* 2013; 526:411–428. <https://doi.org/10.1016/j.gene.2013.05.051> PMID: 23747399
18. Sugiyama A, Shitan N, Sato S, Nakamura Y, Tabata S, Yazaki K. Genome-wide analysis of ATP-binding cassette (ABC) proteins in a model legume plant, *Lotus japonicus*: comparison with Arabidopsis ABC protein family. *DNA Res.* 2006; 13:205–228. <https://doi.org/10.1093/dnares/dsl013> PMID: 17164256
19. Çakır B, Kılıçkaya O. Whole-genome survey of the putative ATP-binding cassette transporter family genes in *Vitis vinifera*. *PLoS One.* 2013; 8:e78860. PMID: PMC3823996 <https://doi.org/10.1371/journal.pone.0078860>
20. Chen P, Li Y, Zhao L, Hou Z, Yan M, Hu B, et al. Genome-wide identification and expression profiling of ATP-binding cassette (ABC) transporter gene family in pineapple (*Ananas comosus*) reveal the role of AcABCG38 in pollen development. *Front Plant Sci.* 2017; 8:2150. PMID: PMC5742209 <https://doi.org/10.3389/fpls.2017.02150> PMID: 29312399
21. Fernandez-Pozo N, Menda N, Edwards JD, Saha S, Teclé IY, Strickler SR, et al. The Sol Genomics Network (SGN)-from genotype to phenotype to breeding. *Nucleic Acids Res.* 2015; 43:1036–1041. <https://doi.org/10.1093/nar/gku1195> PMID: PMC4383978
22. D'Agostino N, Aversano M, Frusciantè L, Chiusano ML. TomatEST database: in silico exploitation of EST data to explore expression patterns in tomato species. *Nucleic Acids Res.* 2007; 35:901–905. <https://doi.org/10.1093/nar/gkl921> PMID: PMC1669777
23. Kudo T, Kobayashi M, Terashima S, Katayama M, Ozaki S, Kanno M, et al. TOMATOMICS: a web database for integrated omics information in tomato. *Plant Cell Physiol.* 2017; 58:1–12. PMID: PMC5444566 <https://doi.org/10.1093/pcp/pcw227>
24. Yano K, Aoki K, Shibata D. Genomic databases for tomato. *Plant Biotechnol.* 2007; 24:17–25. <https://doi.org/10.5511/plantbiotechnology.24.17>
25. Matas AJ, Yeats TH, Buda GJ, Zheng Y, Chatterjee S, Tohge T, et al. Tissue- and cell-type specific transcriptome profiling of expanding tomato fruit provides insights into metabolic and regulatory specialization and cuticle formation. *Plant Cell.* 2011; 23:3893–3910. PMID: PMC3246317 <https://doi.org/10.1105/tpc.111.091173> PMID: 22045915
26. Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature.* 2012; 485:635–641. PMID: PMC3378239 <https://doi.org/10.1038/nature11119> PMID: 22660326
27. Fernandez-Pozo N, Zheng Y, Snyder SI, Nicolas P, Shinozaki Y, Fei Z, et al. The tomato expression atlas. *Bioinformatics.* 2017; 33:2397–2398. PMID: PMC5860121 <https://doi.org/10.1093/bioinformatics/btx190> PMID: 28379331
28. Moco S, Bino RJ, Vorst O, Verhoeven HA, de Groot J, van Beek TA, et al. A liquid chromatography-mass spectrometry-based metabolome database for tomato. *Plant Physiol.* 2006; 141:1205–1218. PMID: PMC1533921 <https://doi.org/10.1104/pp.106.078428> PMID: 16896233
29. Meissner R, Jacobson Y, Melamed S, Levyatuv S, Shalev G, Ashri A, et al. A new model system for tomato genetics. *Plant J.* 1997; 12:1465–1472. <https://doi.org/10.1046/j.1365-313x.1997.12061465.x>
30. Andolfo G, Ruocco M, Di Donato A, Frusciantè L, Lorito M, Scala F, et al. Genetic variability and evolutionary diversification of membrane ABC transporters in plants. *BMC Plant Biol.* 2015; 15:1–15. <https://doi.org/10.1186/s12870-014-0410-4>
31. Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, et al. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Res.* 2017; 45:200–203. <https://doi.org/10.1093/nar/gkw1129> PMID: 27899674
32. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. *Bioinformatics.* 2007; 23:2947–2948. <https://doi.org/10.1093/bioinformatics/btm404> PMID: 17846036

33. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007; 24:1596–1599. <https://doi.org/10.1093/molbev/msm092> PMID: 17488738
34. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, et al. Pfam: the protein families database. *Nucleic Acids Res.* 2014; 42:222–230. <https://doi.org/10.1093/nar/gkt1223> PMCID: PMC3965110
35. Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, et al. Plant ABC proteins—a unified nomenclature and updated inventory. *Trends Plant Sci.* 2008; 13:151–159. <https://doi.org/10.1016/j.tplants.2008.02.001> PMID: 18299247
36. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res.* 2001; 11:1156–1166. <https://doi.org/10.1101/gr.184901> PMID: 11435397
37. Reuscher S, Akiyama M, Mori C, Aoki K, Shibata D, Shiratake K. Genome-wide identification and expression analysis of aquaporins in tomato. *PLoS One.* 2013; 8:e79052. PMCID: PMC3834038 <https://doi.org/10.1371/journal.pone.0079052> PMID: 24260152
38. Reuscher S, Akiyama M, Yasuda T, Makino H, Aoki K, Shibata D, et al. The sugar transporter inventory of tomato: genome-wide identification and expression analysis. *Plant Cell Physiol.* 2014; 55:1123–1141. <https://doi.org/10.1093/pcp/pcu052> PMID: 24833026
39. Martinoia E, Klein M, Geisler M, Bovet L, Forestier C, Kolkusaoglu Ü, et al. Multifunctionality of plant ABC transporters—more than just detoxifiers. *Planta* 2002; 214:345–355. PMID: 11855639
40. Linton KJ, Higgins CF. Structure and function of ABC transporters: the ATP switch provides flexible control. *Pflugers Arch.* 2007; 453:555–567. <https://doi.org/10.1007/s00424-006-0126-x> PMID: 16937116
41. Kaminski WE, Piehler A, Wenzel JJ. ABC A-subfamily transporters: structure, function and disease. *Biochim Biophys Acta.* 2006; 1762:510–524. <https://doi.org/10.1016/j.bbadis.2006.01.011> PMID: 16540294
42. Maathuis FJ, Filatov V, Herzyk P, Krijger GC, Axelsen KB, Chen S, et al. Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. *Plant J.* 2003; 35:675–692. <https://doi.org/10.1046/j.1365-313X.2003.01839.x> PMID: 12969422
43. Blakeslee JJ, Bandyopadhyay A, Lee OR, Mravec J, Titapiwatanakun B, Makam SN, et al. Interactions among PIN-FORMED and P-glycoprotein auxin transporters in Arabidopsis. *Plant Cell.* 2007; 19:131–147. PMCID:1820964 <https://doi.org/10.1105/tpc.106.040782> PMID: 17237354
44. Lee M, Choi Y, Burla B, Kim YY, Jeon B, Maeshima M, et al. The ABC transporter *AtABC14* is a malate importer and modulates stomatal response to CO₂. *Nat Cell Biol.* 2008; 10:1217–1223. <https://doi.org/10.1038/ncb1782> PMID: 18776898
45. Chen S, Sánchez-Fernández R, Lyver ER, Dancis A, Rea PA. Functional characterization of AtATM1, AtATM2, and AtATM3, a subfamily of Arabidopsis half-molecule ATP-binding cassette transporters implicated in iron homeostasis. *J Biol Chem.* 2007; 282:21561–21571. <https://doi.org/10.1074/jbc.M702383200> PMID: 17517886
46. Rea PA. Plant ATP-binding cassette transporters. *Annu Rev Plant Biol.* 2007; 58:347–375. <https://doi.org/10.1146/annurev.arplant.57.032905.105406> PMID: 17263663
47. Ferro M, Brugière S, Salvi D, Seigneurin-Berny D, Court M, Moyet L, et al. AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins. *Mol Cell Proteomics.* 2010; 9:1063–1084. PMCID: PMC2877971 <https://doi.org/10.1074/mcp.M900325-MCP200> PMID: 20061580
48. Jaquinod M, Villiers F, Kieffer-Jaquinod S, Hugouvieux V, Bruley C, Garin J, et al. A proteomics dissection of *Arabidopsis thaliana* vacuoles isolated from cell culture. *Mol Cell Proteomics.* 2007; 6:394–412. PMCID: PMC2391258 <https://doi.org/10.1074/mcp.M600250-MCP200> PMID: 17151019
49. Tarling EJ, de Aguiar Vallim TQ, Edwards PA. Role of ABC transporters in lipid transport and human disease. *Trends Endocrinol Metab.* 2013; 24:342–350. PMCID: PMC3659191 <https://doi.org/10.1016/j.tem.2013.01.006> PMID: 23415156
50. Glavinas H, Krajcsi P, Cserepes J, Sarkadi B. The role of ABC transporters in drug resistance, metabolism and toxicity. *Curr Drug Deliv.* 2004; 1:27–42. PMID: 16305368
51. Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, Bandyopadhyay A, et al. Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. *Plant J.* 2005; 44:179–194. <https://doi.org/10.1111/j.1365-313X.2005.02519.x> PMID: 16212599
52. Santelia D, Vincenzetti V, Azzarello E, Bovet L, Fukao Y, Duchtig P, et al. MDR-like ABC transporter AtPGP4 is involved in auxin-mediated lateral root and root hair development. *FEBS Lett.* 2005; 579:5399–5406. <https://doi.org/10.1016/j.febslet.2005.08.061> PMID: 16198350
53. Kaneda M, Schuetz M, Lin BSP, Chanis C, Hamberger B, Western TL, et al. ABC transporters coordinately expressed during lignification of Arabidopsis stems include a set of ABCBs associated with auxin

- transport. *J Exp Bot.* 2011; 62:2063–2077. PMID: PMC3060696 <https://doi.org/10.1093/jxb/erq416> PMID: 21239383
54. Wu G, Cameron JN, Ljung K, Spalding EP. A role for ABCB19-mediated polar auxin transport in seedling photomorphogenesis mediated by cryptochrome 1 and phytochrome B. *Plant J.* 2010; 62:179–191. <https://doi.org/10.1111/j.1365-3113.2010.04137.x> PMID: 20088903
 55. Kamimoto Y, Terasaka K, Hamamoto M, Takanashi K, Fukuda S, Shitan N, et al. Arabidopsis ABCB21 is a facultative auxin importer/exporter regulated by cytoplasmic auxin concentration. *Plant Cell Physiol.* 2012; 53:2090–2100. <https://doi.org/10.1093/pcp/pcs149> PMID: 23147222
 56. Bernard DG, Cheng Y, Zhao Y, Balk J. An allelic mutant series of ATM3 reveals its key role in the biogenesis of cytosolic iron-sulfur proteins in Arabidopsis. *Plant Physiol.* 2009; 151:590–602. PMID: PMC2754654 <https://doi.org/10.1104/pp.109.143651> PMID: 19710232
 57. Schaedler TA, Thornton JD, Kruse I, Schwarzländer M, Meyer AJ, Van Veen HW, et al. A conserved mitochondrial ATP-binding cassette transporter exports glutathione polysulfide for cytosolic metal cofactor assembly. *J Biol Chem.* 2014; 289:23264–23274. PMID: PMC4156053 <https://doi.org/10.1074/jbc.M114.553438> PMID: 25006243
 58. Davies TGE, Theodoulou FL, Hallahan D L, Hallahan DL, Forde BG. Cloning and characterization of a novel p-glycoprotein homologue from barley. *Gene* 1997; 199:195–202. [https://doi.org/10.1016/S0378-1119\(97\)00367-3](https://doi.org/10.1016/S0378-1119(97)00367-3) PMID: 9358056
 59. Larsen PB, Cancel J, Rounds M, Ochoa V. Arabidopsis *ALS₁* encodes a root tip and stele localized half type ABC transporter required for root growth in an aluminum toxic environment. *Planta.* 2007; 225:1447–1458. <https://doi.org/10.1007/s00425-006-0452-4> PMID: 17171374
 60. Shitan N, Bazin I, Dan K, Obata K, Kigawa K, Ueda K, et al. Involvement of CjMDR1, a plant multidrug-resistance-type ATP-binding cassette protein, in alkaloid transport in *Coptis japonica*. *Proc Natl Acad Sci USA.* 2003; 100:751–756. PMID: PMC141068 <https://doi.org/10.1073/pnas.0134257100> PMID: 12524452
 61. Sasaki T, Ezaki B, Matsumoto H. A gene encoding multidrug resistance (MDR)-like protein is induced by aluminium and inhibitors of calcium flux in wheat. *Plant Cell Physiol.* 2002; 43:177–185. PMID: 11867697
 62. Hanikenne M, Motte P, Wu MCS, Wang T, Loppes R, Matagne RF. A mitochondrial half-size ABC transporter is involved in cadmium tolerance in *Chlamydomonas reinhardtii*. *Plant, Cell and Environment.* 2005; 28:863–873. <https://doi.org/10.1111/j.1365-3040.2005.01335.x>
 63. Remy E, Duque P. Beyond cellular detoxification: a plethora of physiological roles for MDR transporter homologs in plants. *Front Physiol.* 2014; 30:201. PMID: PMC4038776
 64. Nagy R, Grob H, Weder B, Green P, Klein M, Frelet-Barrand A, et al. The Arabidopsis ATP-binding cassette protein AtMRP5/AtABCC5 is a high affinity inositol hexakisphosphate transporter involved in guard cell signaling and phytate storage. *J Biol Chem.* 2009; 284:33614–33622. PMID: PMC2785203 <https://doi.org/10.1074/jbc.M109.030247> PMID: 19797057
 65. Badone FC, Cassani E, Landoni M, Doria E, Panzeri D, Lago C, et al. The low phytic acid1-241 (*pa1-241*) maize mutation alters the accumulation of anthocyanin pigment in the kernel. *Planta.* 2010; 231:1189–1199. <https://doi.org/10.1007/s00425-010-1123-z> PMID: 20191364
 66. Tagashira Y, Shimizu T, Miyamoto M, Nishida S, Yoshida K. Overexpression of a gene involved in phytic acid biosynthesis substantially increases phytic acid and total phosphorus in rice seeds. *Plants.* 2015; 4:196–208. PMID: PMC4844318 <https://doi.org/10.3390/plants4020196> PMID: 27135323
 67. Wanke D, Kolukisaoglu HU. An update on the ABC transporter family in plants: many genes, many proteins, but how many functions? *Plant Biol.* 2010; 1:15–25 <https://doi.org/10.1111/j.1438-8677.2010.00380.x>
 68. Gaillard S, Jacquet H, Vavasseur A, Leonhardt N, Forestier C. AtMRP6/AtABCC6, an ATP-Binding Cassette transporter gene expressed during early steps of seedling development and up-regulated by cadmium in *Arabidopsis thaliana*. *BMC Plant Biol.* 2008; 8:22. PMID: PMC2291051 <https://doi.org/10.1186/1471-2229-8-22> PMID: 18307782
 69. Brunetti P, Zanella L, De Paolis A, Di Litta D, Cecchetti V, Falasca G, et al. Cadmium-inducibile expression of the ABC-type transporter AtABCC3 increases phytochelatin-mediated cadmium tolerance in Arabidopsis. *J Exp Bot.* 2015; 66:3815–3829. PMID: PMC4473984 <https://doi.org/10.1093/jxb/erv185> PMID: 25900618
 70. Hayashi M, Nito K, Takei-Hoshi R, Yagi M, Kondo M, Suenaga A, et al. Ped3p is a peroxisomal ATP-binding cassette transporter that might supply substrates for fatty acid beta-oxidation. *Plant Cell Physiol.* 2002; 43:1–11. PMID: 11828016
 71. Zolman BK, Silva ID, Bartel B. The Arabidopsis *pxa1* mutant is defective in an ATP-binding cassette transporter-like protein required for peroxisomal fatty acid beta-oxidation. *Plant Physiol.* 2001; 127:1266–1278. PMID: PMC129294 PMID: 11706205

72. Bussell JD, Reichelt M, Wiszniewski AAG, Gershenzon J, Smith SM. Peroxisomal ATP-binding cassette transporter *COMATOSE* and the multifunctional protein *ABNORMAL INFLORESCENCE MERISTEM* are required for the production of benzoylated metabolites in *Arabidopsis* seeds. *Plant Physiol.* 2014; 164:48–54. PMID: PMC3875823 <https://doi.org/10.1104/pp.113.229807> PMID: 24254312
73. Dave A, Hernández ML, He Z, Andriotis VME, Vaistij FE, Larson TR, et al. 12-oxo-phytodienoic acid accumulation during seed development represses seed germination in *Arabidopsis*. *Plant Cell.* 2011; 23:583–599. PMID: PMC3077774 <https://doi.org/10.1105/tpc.110.081489> PMID: 21335376
74. Theodoulou FL, Job K, Slocombe SP, Footitt S, Holdsworth M, Baker A, et al. Jasmonic acid levels are reduced in *COMATOSE* ATP-binding cassette transporter mutants. Implications for transport of jasmonate precursors into peroxisomes. *Plant Physiol.* 2005; 137:835–840. PMID: PMC1065384 <https://doi.org/10.1104/pp.105.059352> PMID: 15761209
75. Russell L, Larner V, Kurup S, Bougourd S, Holdsworth M. The *Arabidopsis COMATOSE* locus regulates germination potential. *Development.* 2000; 127:3759–3767. PMID: 10934020
76. Footitt S, Dietrich D, Fait A, Fernie AR, Holdsworth MJ, Baker A, et al. The *COMATOSE* ATP-binding cassette transporter is required for full fertility in *Arabidopsis*. *Plant Physiol.* 2007; 144:1467–1480. PMID: <https://doi.org/10.1104/pp.107.099903> PMID: 17468211
77. Dong J, Lai R, Nielsen K, Fekete CA, Qiu H, Hinnebusch AG. The essential ATP-binding cassette protein RLI1 functions in translation by promoting preinitiation complex assembly. *J Biol Chem.* 2004; 279:42157–42168. <https://doi.org/10.1074/jbc.M404502200> PMID: 15277527
78. Braz AS, Finnegan J, Waterhouse P, Margis R. A plant orthologue of RNase L inhibitor (RLI) is induced in plants showing RNA interference. *J Mol Evol.* 2004; 59:20–30. <https://doi.org/10.1007/s00239-004-2600-4> PMID: 15383904
79. Sarmiento C, Nigul L, Kazantseva J, Buschmann M, Truve E. AtRLI2 is an endogenous suppressor of RNA silencing. *Plant Mol Biol.* 2006; 61:153–163. <https://doi.org/10.1007/s11103-005-0001-8> PMID: 16786298
80. Marton MJ, Vazquez de Aldana CR, Qiu H, Chakraborty K, Hinnebusch AG. Evidence that GCN1 and GCN2, translational regulators of GCN4, function on elongating ribosomes in activation of eIF2 α kinase GCN2. *Mol Cell Biol.* 1997; 17:4474–4489. PMID: 9234705
81. Kato T, Tabata S, Sato S. Analyses of expression and phenotypes of knockout lines for *Arabidopsis* ABCF subfamily members. *Plant Biotechnol.* 2009; 26:409–414. <https://doi.org/10.5511/plantbiotechnology.26.409>
82. Kerr ID, Haider AJ, Gelissen IC. The ABCG family of membrane-associated transporters: you don't have to be big to be mighty. *Br J Pharmacol.* 2011; 164:1767–1779. PMID: PMC3246702 <https://doi.org/10.1111/j.1476-5381.2010.01177.x> PMID: 21175590
83. Banasiak J, Jasinski M. Defence, symbiosis and ABCG transporters. Geisler M, editor. *Plant ABC Transporters, Signaling and Communication in Plants 22*. Springer International Publishing, Cham; 2014. p. 163–184.
84. Bienert MD, Baijot A, Boutry M. ABCG transporters and their role in the biotic stress response. Geisler M, editor. *Plant ABC Transporters, Signaling and Communication in Plants 22*. Springer International Publishing, Cham; 2014. p. 137–162.
85. Bessire M, Borel S, Fabre G, Carraça L, Efremova N, Yephremov A, et al. A member of the PLEIOTROPIC DRUG RESISTANCE family of ATP binding cassette transporters is required for the formation of a functional cuticle in *Arabidopsis*. *Plant Cell.* 2011; 23:1958–1970. PMID: PMC3123938 <https://doi.org/10.1105/tpc.111.083121> PMID: 21628525
86. Chen G, Komatsuda T, Ma JF, Nawrath C, Pourkheirandish M, Tagiri A, et al. An ATP-binding cassette subfamily G full transporter is essential for the retention of leaf water in both wild barley and rice. *Proc Natl Acad Sci USA.* 2011; 108:12354–12359. PMID: PMC3145689 <https://doi.org/10.1073/pnas.1108444108> PMID: 21737747
87. Bultreys A, Trombik T, Drozak A, Boutry M. *Nicotiana plumbaginifolia* plants silenced for the ATP-binding cassette transporter gene *NpPDR1* show increased susceptibility to a group of fungal and oomycete pathogens. *Mol Plant Pathol.* 2009; 10:651–663. <https://doi.org/10.1111/j.1364-3703.2009.00562.x> PMID: 19694955
88. van den Brûle S, Müller A, Fleming AJ, Smart CC. The ABC transporter SpTUR2 confers resistance to the antifungal diterpene sclareol. *Plant J.* 2002; 30:649–662. <https://doi.org/10.1046/j.1365-313X.2002.01321.x>
89. Bird D, Beisson F, Brigham A, Shin J, Greer S, Jetter R, et al. Characterization of *Arabidopsis* ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. *Plant J.* 2007; 52:485–498. <https://doi.org/10.1111/j.1365-313X.2007.03252.x> PMID: 17727615

90. Pighin JA, Zheng H, Balakshin LJ, Goodman IP, Western TL, Jetter R, et al. Plant cuticular lipid export requires an ABC transporter. *Science*. 2004; 306:702–704. <https://doi.org/10.1126/science.1102331> PMID: 15499022
91. Panikashvili D, Savaldi-Goldstein S, Mandel T, Yifhar T, Franke RB, Hofer R, et al. The Arabidopsis *DESPERADO/AtWBC11* transporter is required for cutin and wax secretion. *Plant Physiol*. 2007; 145:1345–1360. PMID: PMC2151707 <https://doi.org/10.1104/pp.107.105676> PMID: 17951461
92. Kuromori T, Fujita M, Urano K, Tanabata T, Sugimoto E, Shinozaki K. Overexpression of *AtABCG25* enhances the abscisic acid signal in guard cells and improves plant water use efficiency. *Plant Science*. 2016; 251:75–81. <https://doi.org/10.1016/j.plantsci.2016.02.019> PMID: 27593465
93. Kuromori T, Ito T, Sugimoto E, Shinozaki K. Arabidopsis mutant of *AtABCG26*, an ABC transporter gene, is defective in pollen maturation. *J Plant Physiol*. 2011; 168:2001–5. <https://doi.org/10.1016/j.jplph.2011.05.014> PMID: 21696844
94. Zhu YQ, Xu KX, Luo B, Wang JW, Chen JW. An ATP-binding cassette transporter GhWBC1 from elongating cotton fibers. *Plant Physiol*. 2003; 133:580–588. <https://doi.org/10.1104/pp.103.027052> PMID: 12972649
95. Rayapuram N, Hagenmuller J, Grienenberger JM, Giegé P, Bonnard G. AtCCMA interacts with AtCcmB to form a novel mitochondrial ABC transporter involved in cytochrome C maturation in Arabidopsis. *J Biol Chem*. 2007; 282:21015–21023. <https://doi.org/10.1074/jbc.M704091200> PMID: 17550895
96. Xu XM, Møller SG. *AtNAP7* is a plastidic SufC-like ATP-binding cassette/ATPase essential for Arabidopsis embryogenesis. *Proc Natl Acad Sci USA*. 2004; 101:9143–9148. <https://doi.org/10.1073/pnas.0400799101> PMID: 15184673
97. Yazaki K, Shitan N, Sugiyama A, Takanashi K. ATP-binding cassette proteins in plants. *Int Rev Cell Mol Biol*. 2009; 276:263–299. [https://doi.org/10.1016/S1937-6448\(09\)76006-X](https://doi.org/10.1016/S1937-6448(09)76006-X) PMID: 19584015
98. Nagashima A, Uehara Y, Sakai T. The ABC subfamily B auxin transporter *AtABCB19* is involved in the inhibitory effects of *N*-1-naphthylphthalamic acid on the phototropic and gravitropic responses of Arabidopsis hypocotyls. *Plant Cell Physiol*. 2008; 49:1250–1255. <https://doi.org/10.1093/pcp/pcn092> PMID: 18556728