PLOS ONE

RESEARCH ARTICLE

Genome-wide identification and gene expression pattern of ABC transporter gene family in *Capsicum* spp.

Carlos Lopez-Ortiz[1☯], Sudip Kumar Dutta[1,2☯], Purushothaman Natarajan[1,3], Yadira Peña-Garcia[1], Venkata Abburi[1], Thangasamy Saminathan[1], Padma Nimmakayala[1], Umesh K. Reddy[1]*

1 Department of Biology, Gus R. Douglass Institute, West Virginia State University, Institute, West Virginia, United States of America, **2** ICAR RC NEH Region, Mizoram Centre, Kolasib, Mizoram, India, **3** Department of Genetic Engineering, School of Bioengineering, SRM Institute of Science and Technology, Kattankulathur, India

☯ These authors contributed equally to this work.
* ureddy@wvstateu.edu

OPEN ACCESS

Citation: Lopez-Ortiz C, Dutta SK, Natarajan P, Peña-Garcia Y, Abburi V, Saminathan T, et al. (2019) Genome-wide identification and gene expression pattern of ABC transporter gene family in *Capsicum* spp.. PLoS ONE 14(4): e0215901. https://doi.org/10.1371/journal.pone.0215901

Editor: Rajesh Mehrotra, Birla Institute of Technology and Science, INDIA

Received: July 12, 2018

Accepted: April 10, 2019

Published: April 30, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files. The Illumina reads for transcriptome analysis performed in this study were deposited with the Sequence Reads Archive (NCBI) under the following accession number PRJNA526219 (https://www.ncbi.nlm.nih.gov/sra/PRJNA526219).

Funding: This study was supported by the National Institute of Food and Agriculture (USDA-NIFA) (grant no. 2016-06616 to PN). The funders had no role in study design, data collection and analysis,

Abstract

ATP-binding cassette (ABC) transporter genes act as transporters for different molecules across biological membranes and are involved in a diverse range of biological processes. In this study, we performed a genome-wide identification and expression analysis of genes encoding ABC transporter proteins in three *Capsicum* species, i.e., *Capsicum annuum*, *Capsicum baccatum* and *Capsicum chinense*. *Capsicum* is a valuable horticultural crop worldwide as an important constituent of many foods while containing several medicinal compounds including capsaicin and dihydrocapsaicin. Our results identified the presence of a total of 200, 185 and 187 ABC transporter genes in *C. annuum*, *C. baccatum* and *C. chinense* genomes, respectively. Capsaicin and dihydrocapsaicin content were determined in green pepper fruits (16 dpa). Additionally, we conducted different bioinformatics analyses including ABC genes classification, gene chromosomal location, *Cis* elements, conserved motifs identification and gene ontology classification, as well as profile expression of selected genes. Based on phylogenetic analysis and domain organization, the *Capsicum* ABC gene family was grouped into eight subfamilies. Among them, members within the ABCG, ABCB and ABCC subfamilies were the most abundant, while ABCD and ABCE subfamilies were less abundant throughout all species. ABC members within the same subfamily showed similar motif composition. Furthermore, common *cis*-elements involved in the transcriptional regulation were also identified in the promoter regions of all *Capsicum* ABC genes. Gene expression data from RNAseq and reverse transcription-semi-quantitative PCR analysis revealed development-specific stage expression profiles in placenta tissues. It suggests that ABC transporters, specifically the ABCC and ABCG subfamilies, may be playing important roles in the transport of secondary metabolites such as capsaicin and dihydrocapsaicin to the placenta vacuoles, effecting on their content in pepper fruits. Our results provide a more comprehensive understanding of ABC transporter gene family in different *Capsicum* species while allowing the identification of important candidate genes related to capsaicin content for subsequent functional validation.

decision to publish, or preparation of the manuscript.

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

Introduction

Pepper (*Capsicum* spp.) is a member of the Solanaceae family and is closely related to potato, tomato, eggplant, tobacco and petunia. Pepper represents an important horticultural crop worldwide not only because of its economic importance, but also due to its medicinal value. Moreover, pepper fruits have been widely used as a coloring agent, food flavoring, cosmetic and pharmaceutical ingredient, and as an ornamental product. It also has nutrimental value by containing vitamins A, B, C, and E, as well as phytochemicals such as phenolic compounds, carotenoids, and capsaicin. In addition to dietary and culinary importance, capsaicinoid compounds (capsaicin and dihydrocapsaicin) of pepper have a beneficial effect for humans, including antioxidant, anticarcinogenic, antimutagenic, antiaging, and antibacterial properties [1–4].

Capsaicinoids are strongly pungent alkaloids that accumulate in the placenta of maturing *Capsicum* fruits. Pepper genotypes exhibit a wide range of capsaicinoid accumulation as a result of both environmental and genetic variability. Recently, Nimmakayala et al. [5] determined that the major markers linked to capsaicinoid synthesis are ankyrin-like protein, the IKI3 protein family and the ATP-binding cassette (ABC) transporter family, which suggests that their activity may be involved in the pungency modulations in pepper. The ABC transporter gene family represents one of the largest gene families, with a transporter activity that is conserved and ubiquitous in all living organisms [6, 7]. Most ABC transporter proteins that have been characterized are ATP-dependent membrane-bound transporters that are able to translocate a wide range of molecules such as lipids, proteins, chemotherapeutic drugs and heavy metals via intra- and extracellular membranes [8]. In addition, some ABC proteins also act as regulators of ion channels, receptors and proteins involved in mRNA translation and ribosome biogenesis [9].

Eukaryotes feature three common arrangements of ABC transporters: full-sized transporters composed of two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs), half-sized transporters containing one TMD and one NBD, and a third type with no TMDs but two NBDs [10]. The NBD is present in all three structural types and contains many key conserved motifs: Walker A, Q-loop, Walker B, D-loop, switch H-loop, and a signature motif (LSGGQ) that is exclusively found in ABC proteins, which distinguishes ABC proteins from other ATPases [11, 12].

Plant ABC transporters are divided into eight subfamilies—A, B, C, D, E, F, G, and I—based on their protein solubility, presence of TMDs, function, and amino acid sequence [10, 13]. Although there is a ninth subfamily group, the ABCH subfamily, it has not been identified in plants. Several ABC transporters genes have been characterized in plants, however, most of them were first identified as transporters contributing to detoxification processes [14]. Subsequently, the release of genomic data and development of bioinformatics analyses have led to comprehensive research on the identification of new functions, as well as the characterization of new ABC transporter gene family in diverse plants such as *Arabidopsis*, *Oryza sativa* and *Lotus japonicus* [13, 15–17], *Zea mays* [18, 19], *Brassica rapa* [20], *Brassica napus* [21] and *Vitis vinifera* [22]. Thus, apart from just detoxification functions, the understanding of several novel roles of the ABC transporter genes have been revealed. To date, it has reported that plant ABC transporters are involved in many important physiological, growth and developmental processes. Furthermore, the transport substrates of plant ABCs are divergent and include conjugated compounds, phytohormones, primary products, lipids and lipophilic compounds [23–25].

The recent release of the genome sequence of pepper [2] has provided a platform for genome-wide analysis that allow the identification and characterization of entire gene families present in hot peppers [26–28]. For instance, it makes feasible the performance of deeper studies in the ABC transporter gene family, which remains poorly understood in *Capsicum* spp. In

the current study, we report a genome-wide identification and characterization of ABC transporter genes in three *Capsicum* species (i.e., *C. annuum*, *C. baccatum* and *C. chinense*) including sequence alignment, phylogenetic analysis, chromosomal location and expression profile of *C. annuum* and *C. chinense*. Our results lay a foundation for further functional characterization of each ABC transporter gene among *Capsicum* species and provide useful information for better understanding the role and evolution of this gene family in higher plants.

Materials and methods

Plant material

C. annuum cv. CM334, *C. baccatum* cv. PBC81 and two varieties of *C. chinense* (Pimenta da neyde and Naga morich) were grown in triplicate samples in an experimental field at West Virginia State University. Fruits at 6, 16 and 25 days post-anthesis (dpa) were collected from all cultivars and stored at -80˚C. Quantitative analysis of capsaicin and dihydrocapsaicin content in green pepper fruits (16 dpa) were determinate with the 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) [5].

Identification of the ABC transporter genes in pepper

To identify all members of the ABC transporter gene family in the pepper genomes, the proteomes for the three *Capsicum* species were downloaded from the pepper genome platform (PGP) (http://passport.pepper.snu.ac.kr/?t=PGENOME) [2]. A local BLASTP search was used to query the full-length amino acid sequences of ABC transporter proteins from *Arabidopsis* (https://phytozome.jgi.doe.gov/pz/portal.html) [29]. All output genes were collected and confirmed by using the software HMMER3.0 [30]. *Capsicum* genes were searched with the PF00005 ABC transporter domain, PF01061 ABC-2 transporter domain and PF00664 ABC transporter transmembrane region domain, the ABC transporter domains were confirmed using the Pfam web server (http://Pfam.sanger.ac.uk/) [31]. Genes with E-value $>$ 1E-05 and redundant genes were excluded. Candidate genes were analyzed in the SMART database (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1) [32] to verify the presence of the NBD and TMD domains. Genes with NBD and TMD domains were considered members of the ABC transporter family in pepper, and the coding sequences (CDS) were downloaded from the PGP database. The Jackhmmer tool (https://www.ebi.ac.uk/Tools/hmmer/search/jackhmmer) [33] was used to classify the ABC transporter gene family in subfamilies by using the UniProt reference proteome database with E-value = 0.01 for sequence matches and 0.03 for hit matches.

Sequence alignment and phylogenetic analysis

The amino acid sequences of the *Capsicum* species and *Arabidopsis* were imported into MEGAX [34] and multiple sequence alignments were carried out using ClustalW [35] with gap-open and gap-extension penalties of 10 and 0.1, respectively. The alignment file was then used to construct a phylogenetic tree based on the neighbor-joining (NJ) method. After bootstrap analysis with 1000 replicates, the tree was displayed by using the interactive Tree Of Life platform (iTOL; http://itol.embl.de/index.shtml) [36].

Chromosomal location, *Cis*-element analysis and identification of conserved motifs

The physical chromosome location data for each ABC transporter was downloaded from the PGP database and mapped onto the 12 chromosomes of pepper by using MapInspect. The

protein size, molecular weight (MW) and theoretical isoelectric point (pI) of each ABC transporter were computed by using the proteome database and sequence analysis tools on the ExPASy Proteomics Server (http://expasy.org/) [37]. For *Cis*-element analysis, all promoter sequences (1,500 bp upstream of initiation codon "ATG") of ABCs were extracted from the pepper genome. Then, the *cis*-regulatory elements of promoters for each gene were identified by using PLACE: A database of plant *cis*-acting regulatory DNA elements (http://www.dna.affrc.go.jp/PLACE/) [38]. Protein sequence motifs were identified by using Multiple Em for Motif Elicitation (MEME) (http://meme-suite.org/tools/meme) [39]. The analysis was performed with maximum number of motifs 10 and optimum width of motif $\geq$50. Discovered MEME motifs were searched in the Expasy-Prosite database with ScanProsite server (https://prosite.expasy.org/scanprosite/) [40].

Gene ontology (GO) annotation and modeling of ABC proteins

The functional annotation of ABC transporters was performed using Blast2GO software (http://www.blast2go.com). The amino acid sequences of ABC genes were imported into Blast2GO program to execute three steps: 1) BLASTp against the NCBI non-redundant protein database, 2) mapping and retrieval of GO terms associated with the BLAST results, and 3) annotation of GO terms associated with each query to relate the sequences to known protein function.

Identification of syntenic ABC paralogs pairs and gene synteny analysis

The syntenic ABC transporter paralogs pairs were identified by searching the gene duplication across all the species with the following criteria: 1) genes with >70% coverage of the alignment length; 2) genes with >70% identity in the aligned region; and 3) a minimum of two duplication events considered for strongly connected genes [41]. For each paralog pair, the non-synonymous substitution rate (Ka), the synonymous substitution rate (Ks) and the ω (= Ka/Ks) of paralog pairs were estimated by using KaKs_Calculator 2.0 [42]. The duplication date of paralog pairs was estimated by the formula T = Ks/2λ, assuming a clock-like rate (λ) of 6.96 synonymous substitutions per 10^{-9} years [43].

Transcriptome sequencing of *C. chinense* green fruits

Green fruits (16 dpa) from two different cultivars of *C. chinense* were used for whole-transcriptome sequencing. Total RNA was isolated from the pooled tissues of three biological replicates for each cultivar with the Plant RNA mini spin kit (Macherey-Nagel). The quantity and quality of the total RNA were analyzed with the Agilent 2100 Bioanalyzer and Qubit 4 Fluorometer (Invitrogen), respectively. The RNA sequencing libraries were prepared by using the NEBNext Ultra II RNA Library Prep Kit according to the manufacturer's protocol. The mRNAs were enriched by using magnetic beads with Oligo (dT), then fragmented into shorter fragments with a fragmentation buffer. The first-strand cDNA was synthesized from the fragmented mRNA with a random hexamer primer. The resulting cDNAs were added to sequencing adapters, and sequencing primers were used for library amplification. The insert size of the library was analyzed with Agilent 2100 Bioanalyzer (Invitrogen), and the Qubit 4 Fluorometer (Invitrogen) was used for library quantification. The RNA sequencing library from each sample was sequenced in the Illumina NextSeq 500 platform with paired-end sequencing. The resulting image files were converted to FASTQ with 2x75-bp reads. The Illumina reads were deposited with the Sequence Reads Archive (NCBI) under the following accession number PRJNA526219.

Analysis of *C. chinense* transcriptome to study ABC transporter genes

The sequencing adapters and low-quality reads (Phred score QV<30) were removed by using cutadapt (https://cutadapt.readthedocs.io/en/stable/guide.html) [44] and sickle (https://github.com/najoshi/sickle) [45] respectively. The quality-filtered reads were mapped to the *C. chinense* reference genome [2] by using the mem algorithm of the BWA tool [46] to generate SAM alignment. The read count table for genes from *C. chinense* was created for all the samples by using the SAM alignment and HTSeq R package [47]. The gene expression based on the read counts were studied by reads per kilobase per million (RPKM). The RPKM values for each gene were calculated based on the read count table, the total number of reads and gene length (kb). The ABC transporters in *C. chinense* (CcABCs) were identified by homology search against the CDS sequences from *C. annuum* by using a BLASTN algorithm (identity $\geq$ 98% and coverage $\geq$ 70%). The gene annotation of the ABC transporter genes identified from *C. chinense* was confirmed by using the BLASTx algorithm against the NCBI non-redundant protein database.

Expression pattern of ABC transporters in *C. annuum* and *C. chinense*

The RNA-seq gene expression data in placenta tissues (6 dpa, 16 dpa, 25 dpa) from *C. annuum* cv. CM334 was retrieved from the RNA-seq data published by [2]. A BLASTN search was performed (identity $\geq$ 98% and coverage $\geq$ 70%) to identify the orthologs genes between *C. annuum* ABC (CaABC) and *C. chinense* (CcABC) transporters. The RPKM expression values for identified CaABC protein genes were extracted from the dataset and a gene expression heatmap was generated for *C. annuum* and *C. chinense* orthologs by using the ClustVis web tool (https://biit.cs.ut.ee/clustvis/) [48].

RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from pepper fruits (6, 16 and 25 dpa) by using the Plant RNA mini spin kit (Macherey-Nagel). First-strand cDNA was synthesized with 1 μg total RNA per sample by using the Super Script First-Strand Synthesis system (Invitrogen). To identify in the three *Capsicum* genomes the orthologs of the markers previously reported by [5] for the ABC transporter family, the CDS sequences for the CA06g14430 and CA11g09150 genes were downloaded from the Sol Genomics database (https://solgenomics.net/) [49] and a BLASTN search was performed (identity $\geq$ 98% and coverage $\geq$ 70%) across the three pepper genomes. Gene-specific primers for the selected *Capsicum* ABC transporter orthologs were designed by using Primer3Plus (http://www.primer3plus.com/). The qRT-PCR analysis involved a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with a total volume of 20 μL containing 1 μL cDNA template, 2 μL forward and reverse primers (10 μM), 10 μL SYBR Green PCR Master (ROX) (Roche, Shanghai) and 7 μL sterile distilled water. For each sample, three replicates were run to compute the average Ct values. The data were analyzed by the $2^{-\Delta\Delta Ct}$ method [50]. Relative gene expression was normalized against that of the endogenous control β-tubulin [51].

Results and discussion

Capsaicin and dihydrocapsaicin content in pepper

Capsaicinoids are responsible for the hot or burning sensation of chili, pungency and flavor are the primary properties of pepper fruits [52]. About 80% to 90% of capsaicinoids in chili fruit is represented by capsaicin and dihydrocapsaicin, and their accumulation occurs over a relatively short period during the latter stages of fruit development [53]. *C. chinense* is one of

the hottest chili peppers in the world; in general, chili species and varieties contain about 1% capsaicin, but this content can range from 2% to 4% [54]. In this study, the highest capsaicin and dihydrocapsaicin content was for *C. chinense* cv. Naga morich, with 14.67 mg g^{-1} and 5.54 mg g^{-1} dry weight (DW) tissue, respectively. On the other hand, 4.62 mg g^{-1} and 1.08 mg g^{-1} DW tissue were reported for *C. chinense* cv. Pimienta da neyde, and 0.823 mg g^{-1} and 0.393 mg g^{-1} DW tissue in *C. annuum* cv. CM334. The lowest value across all the species was for *C. baccatum*, with a content of 0.55 and 0.15 mg g^{-1} for capsaicin and dihydrocapsaicin respectively (Fig 1).

Capsaicinoids biosynthesis is carried out principally in the placental tissues of pepper fruits by the action of several enzymes [55, 56]. Recently, NGS approaches including genotyping by sequencing (GBS), based GWAS and RNAseq analysis of placenta tissues have been used for the identification of novel genes involved in the capsaicinoids biosynthesis pathway. Moreover, these approaches have allowed the study of the mechanisms involved in the pungency modulations in pepper. Liu et al. [57] predicted the function of three novel genes i.e., dihydroxyacid dehydratase (DHAD), threonine deaminase (TD) and prephenate aminotransferase (PAT) which play key roles in the capsaicinoids biosynthetic pathway. In a recent association mapping study carried out by Nimmakayala et al. [5], it was identified significant SNPs associated with capsaicin content and fruit weight. This study revealed that genes such as Ankyrin-like protein, IKI3 family protein, pentatricopeptide repeat protein and ABC transporter G and C subfamilies are important players regulating capsaicin content. The SNPs associated with the ABC transporter gene family were S6_203416571 and S11_83592400 in the locus CA06g14430 and CA11g09150 respectively (Fig 2). Particularly, the SNP S6_203416571 located in chromosome 6, showed a high allelic effect (Fig 2A).

Genome-wide identification of ABC proteins in pepper

To identify the ABC protein family in pepper, we performed a BLASTP search of the three pepper genomes from the PGP database. A total of 572 genes potentially encoding ABC proteins were identified: 200 from *C. annuum* (CaABC), 185 from *C. baccatum* (CbABC), and 187 from *C. chinense* (CcABC) (Table 1). To investigate the evolutionary relationship between *Capsicum* species and *Arabidopsis* ABC transporter proteins (AtABC), we performed phylogenetic analysis of the pepper and *Arabidopsis* ABC proteins. The protein sequences of *Capsicum* ABC genes and AtABC proteins (119 protein sequences containing the ABC transporter

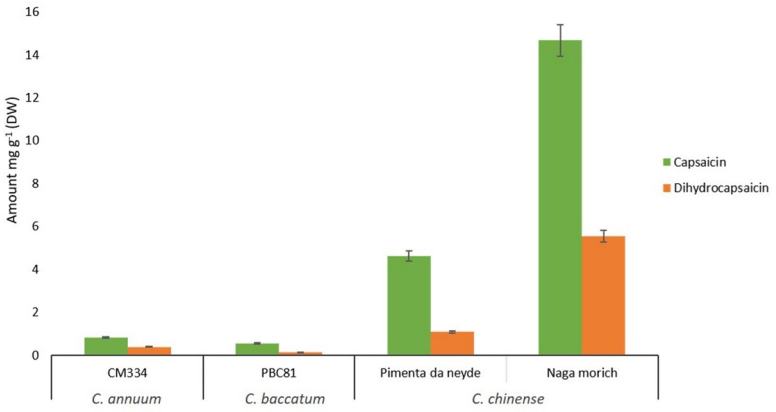

Fig 1. Capsaicin and dihydrocapsaicin content in pepper. Capsaicin and dihydrocapsaicin levels in pepper powder from dried green fruit (16 days post anthesis (dpa)). Values are means ± SD; n = 3.

https://doi.org/10.1371/journal.pone.0215901.g001

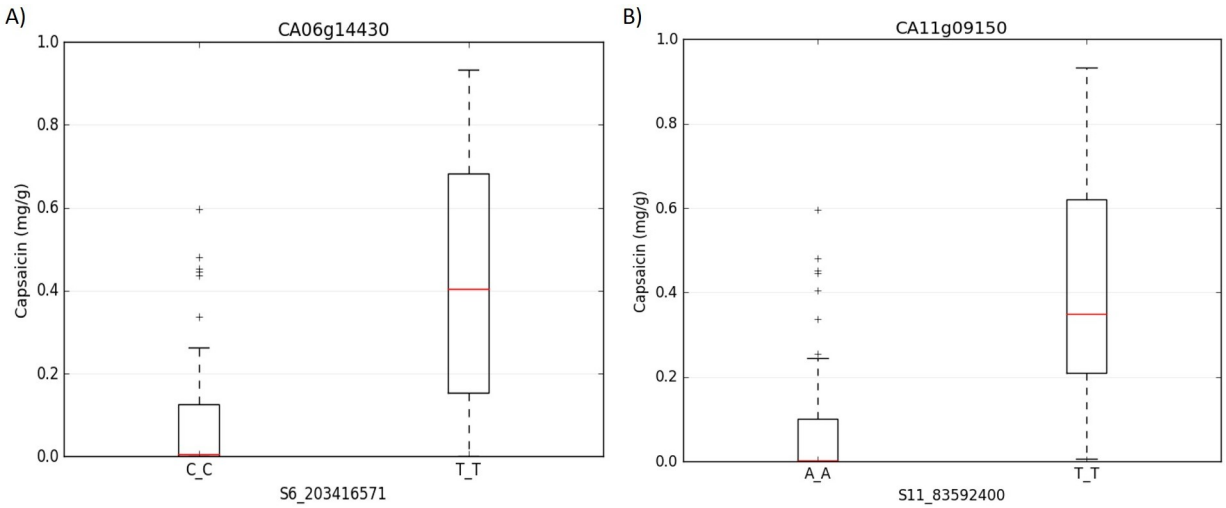

Fig 2. Allelic effect of two significantly associated SNPs markers for capsaicin content in *C. annuum*. Each plot is labeled with the SNP position in the X-axis. Y-axis represents the values for capsaicin levels ($mg \cdot g^{-1}$) in pepper powder from dried green fruit. Boxplot A) shows the effect of SNP marker in locus CA06g14430 on chromosome 06, whereas boxplot B) shows the effect of SNP marker in locus CA11g09150 on chromosome 11.

https://doi.org/10.1371/journal.pone.0215901.g002

domain) were aligned by using MEGAX, and an unrooted phylogenetic tree was constructed by a NJ method with 1000 bootstrap replications (Fig 3).

An extensive research on ABC transporters has resulted in several naming schemes. In most of the cases, the transporters were named on the basis of mutant characteristics. Thus, different names were assigned to the same subfamily or selected members with common characteristics. To conform to plant and animal ABC communities, the Human Genome

Table 1. Comparative analysis of ABC proteins between Capsicum and other plant species.

Species name	ABC transporter subfamilies								Total ABCs transporter	Reference
	ABCA	ABCB	ABCC	ABCD	ABCE	ABCF	ABCG	ABCI		
Arabidopsis thaliana	12	29	15	2	3	5	43	21	130	[13]
Arabidopsis lyrata	12	25	15	2	3	10	43	22	132	[20]
Brassica rapa	11	38	21	2	7	7	63	30	179	
Populus trichocarpa	6	40	29	3	2	4	78	42	204	
Glycine max	8	49	40	10	2	10	113	39	271	
Carica papaya	5	18	13	3	2	4	36	32	113	
Vitis vinifera	5	30	26	1	1	6	71	41	181	
Brachypodium distachyon	6	32	19	4	4	7	44	22	138	
Amborella trichopoda	4	19	17	2	2	5	46	42	137	
Oryza sativa	6	28	17	3	1	6	56	24	141	
Oryza sativa	6	27	17	3	2	6	50	16	127	[16]
Zea mays	6	31	13	4	2	7	54	13	130	[18]
Brassica napus	30	69	47	5	13	14	116	20	314	[21]
Ananas comosus	5	20	16	2	1	5	42	9	100	[58]
Solanum lycopersicum	9	29	26	2	2	6	70	10	154	[59]
Capsicum annuum	10	48	26	2	1	10	95	8	200	
Capsicum baccatum	7	41	24	3	1	6	94	9	185	
Capsicum chinense	9	44	23	5	1	6	91	8	187	

https://doi.org/10.1371/journal.pone.0215901.t001

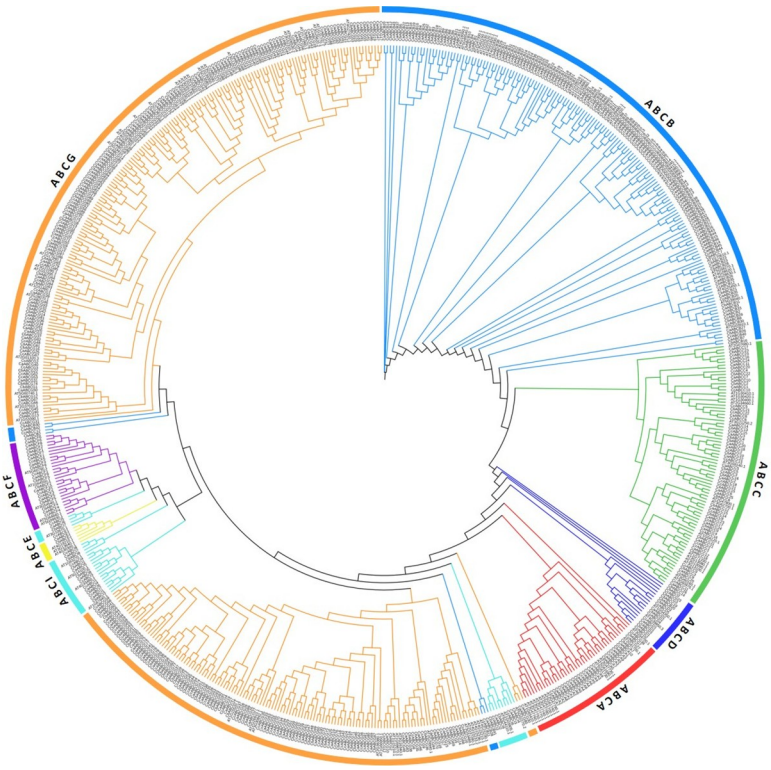

Fig 3. Phylogenetic relationships of *Capsicum* species and *Arabidopsis* ABC transporter proteins. The 572 and 119 ABC proteins identified from Capsicum species and Arabidopsis, respectively, were subjected to phylogenetic analysis by the neighbor-joining method with 1000 bootstrap replicates. Subfamily names (ABCA-I, except ABCH) are indicated by different colors.

https://doi.org/10.1371/journal.pone.0215901.g003

Organization (HUGO) nomenclature system [10] was adopted to designate all putative ABC proteins as ABCA-G and ABCI to all ABC transporter subfamilies. Overall, *Capsicum* ABC proteins followed the same pattern as *Arabidopsis* (Fig 3). Based on phylogenetic association with AtABCs and using the jackHmmer tool, *Capsicum* ABCs were classified into eight subfamilies previously mentioned. The number of members of ABCs within each subfamily in *Capsicum* were similar to other plants such as *Arabidopsis* [13], *B. rapa* [20] and tomato [59]. In order of abundance, ABCG, ABCB and ABCC subfamilies were the most prevalent groups throughout all species, whereas the smallest number of members were in the ABCD and ABCE subfamilies; for this last subfamily, only one member was identified in all the three *Capsicum* species analyzed.

For convenience, the ABC transporters were named CaABC1 to CaABCn for *C. annuum* based on their subfamily group and were classified similarly for the other species. The *Capsicum* ABC proteins vary substantially in size and sequences of their encoded region, as well as in their physicochemical properties across all species. The locations of the ABC domains within the protein also differ. The physical locations, coding sequence length, protein characteristics and topology for ABC transporters identified for each species are in S1–S3 Tables. The domain organizations for ABC transporters are almost as varied as their function: proteins of the ABCA-ABCD subfamilies have a forward direction for domain organization (TMD-NBD), whereas the proteins of the ABCG and ABCH subfamilies contain the reverse domain organization (NBD-TMD). ABCE and ABCF proteins contain only two NBDs and were characterized as soluble proteins. ABCI proteins generally possess only one domain, mainly NBD or

TMD. Topological diversity is one of the unique characteristics of ABC proteins. The ABC transporters are divided in three common arrangements: full-sized transporters, half-sized transporters and a third type that has no TMDs but two NBD domains [10]. A typical full-sized ABC protein consists of ≥1,200 amino acid residues [14]. The 200 CaABC proteins ranged from 52 to 1831 amino acid residues, the CbABCs from 89 to 1864 residues and the CcABCs from 86 to 1965 residues. Nevertheless, it is important to mention that all of them possess at least one NBD, thus, they can be classified as ABC transporters and were included in this study. Some of the pepper ABC proteins with shorter sequences might be thought as pseudogenes or not annotated genes. These shorter sequences were also found in the genome-wide analysis of ABC transporters in tomato, *B*. *rapa* and pineapple [20, 58, 59]. Among the 572 ABC transporters, 212 lack a TMD and were considered soluble ABC proteins. The remaining 360 members possess TMDs and were considered ABC transporters across all species. Overall, 134 *Capsicum* ABC proteins are full-sized proteins possessing (TMD-NBD)x2 domains: 46, 40, 48 for *C*. *annuum*, *C*. *baccatum* and *C*. *chinense*, respectively. Among these members, 22, 24 and 28, respectively, exhibit a forward topology (TMD-NBD), whereas 24, 26, and 20 have a reverse topology (NBD-TMD). In total, 135 ABC transporters were classified as half-sized, having forward (TMD-NBD) or reverse (NBD-TMD) orientations. Among the half-sized *Capsicum* ABC proteins, 26 exhibit a forward and 109 a reverse domain orientation. A total of 233 ABC transporters were considered quarter-sized or single-structure proteins: 184 have an NBD domain, and 49 a TMD domain. *Capsicum* ABC proteins were also classified under an ABC2 (NBD-NBD) structure: 26 have the NBD-NBD structure and 3 the TMD-TMD structure. In total, 37 ABCs were uniquely characterized, with NBD-TMD-NBD, TMD-NBD-TMD and TMD-TMD-NBD-TMD-TMD structures. The differences in the topology domain orientations might have resulted from gene duplication during evolution or evolved to render specific physiological functions under biotic or abiotic stress [60].

Chromosomal locations and syntenic *Capsicum* ABC paralog pairs

A total of 544 (95.1%) ABC transporters were physically mapped on all 12 chromosomes of pepper, and the other 28 genes were located on unanchored scaffolds (Fig 4). ABCG, ABCB and ABCC subfamilies are unevenly distributed across all chromosomes. ABCD (in chromosome 2 and 12) and ABCE (in chromosome 01) subfamilies are the most conserved across all the *Capsicum* species. Among all chromosomes, chromosome 3 of *C*. *annuum* contains the highest number of ABCs—32 (16%)—followed by chromosome 6 (14.5%). Among all species, chromosomes 3, 6 and 12 contain the highest number of ABCs, with the minimum on chromosome 10.

The distribution pattern of ABC transporters on individual chromosomes also indicated certain physical regions with a relatively higher accumulation of multiple ABC gene clusters, such as chromosome 3 and 6 at the lower end of the arms for all species. The distribution of ABC transporters differs among the three genomes. Some ABC gene clusters occur in one species but not in the other genomes; for example, in chromosome 2, ABCs were present in the upper chromosome part in *C*. *annuum* and *C*. *baccatum* but were absent in *C*. *chinense*. On the other hand, ABCG and ABCB family members were found on at the lower part of chromosome 4 in *C*. *chinense* and *C*. *baccatum* but not in *C*. *annuum*. A clear example is at the upper end of chromosome 8, where a cluster of genes corresponding to the ABCB and ABCG members are present in *C*. *chinense*, and only one gene appears in *C*. *baccatum*, but with no presence reported in *C*. *annuum*.

Syntenic paralogs are genes that are located in syntenic fragments. The syntenic paralog pairs were identified between and within the three *Capsicum* genomes. Simultaneously, we

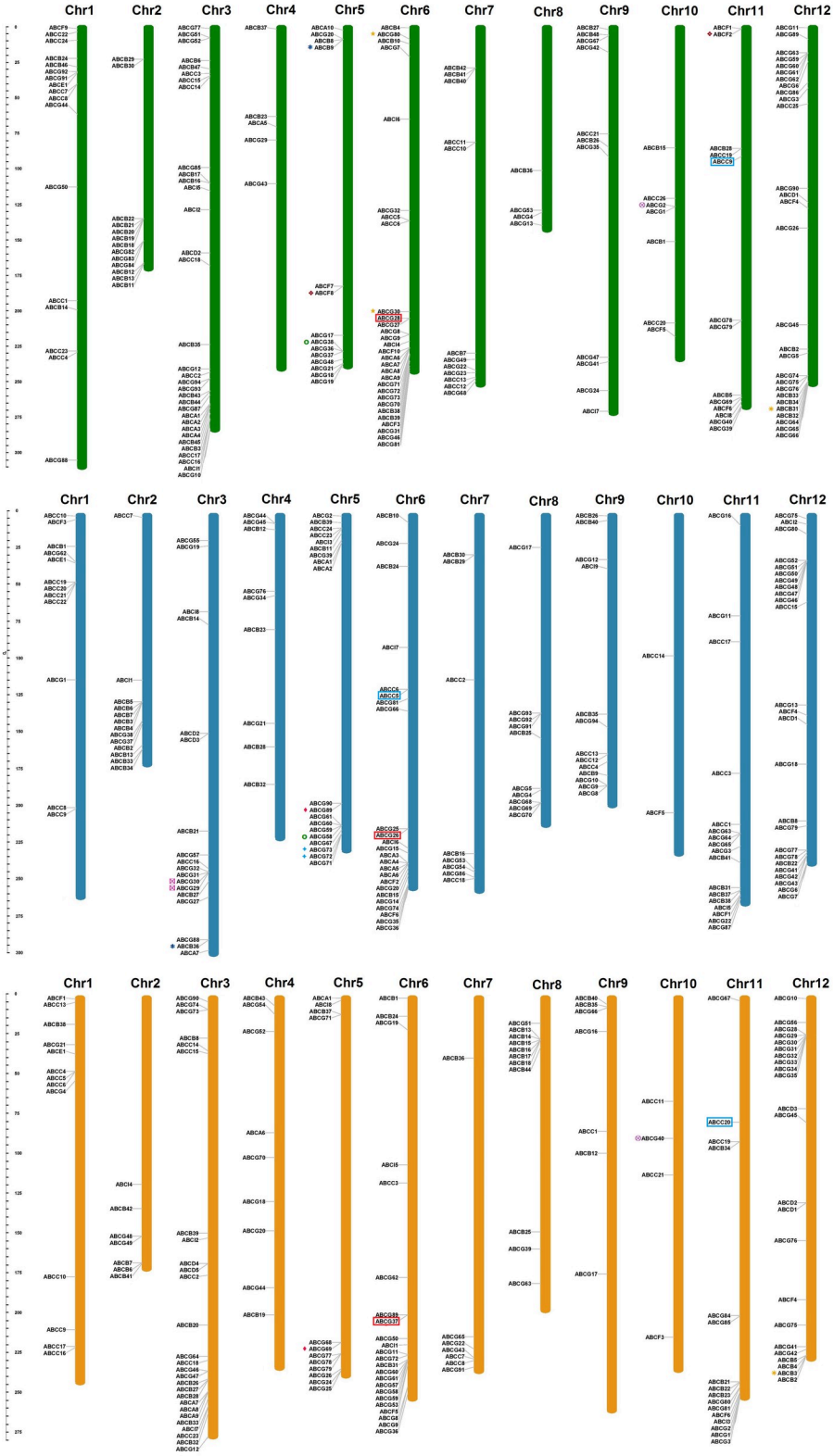

Fig 4. Chromosomal locations of ABC transporter proteins in pepper *C. annuum* (green), *C. baccatum* (blue) and *C. chinense* (orange). Chromosome numbers are represented at the top of each chromosome. The left panel scale

indicates the chromosome length in Mb. The paralogous ABC gene pairs are represented with different colors and shapes. Orthologs genes of CA06g14430 and CA11g09150 are represented by red and blue boxes respectively.

https://doi.org/10.1371/journal.pone.0215901.g004

further identified synonymous (Ks) and non-synonymous (Ka) values to explore the selective pressures on these paralog pairs to understand the expansion of this gene family in pepper. In total, 14 pairs of ABC transporter syntenic paralogs were identified across all species (Table 2). Six paralog pairs were within species and the remaining were intra-species. Among the eight intra-species duplications, three segmental duplication gene pairs were intra-chromosomal, located on chromosomes 5 and 3 for *C. baccatum* and chromosome 6 for *C. annuum*. Only one segmental duplication CaABCF8-CaABCF2 in *C. annuum* involved two different chromosomes. Moreover, the duplicated paralog ABC transporter pairs belong to the same subfamily. The Ka/Ks (ω) ratios for segmental duplications ranged from 0.06 to 1.57, with a mean of 0.81. In total, 11 out of 14 of the paralogs pair were under purifying selection, with ω ratios < 1. The ω ratios for 3 syntenic paralogs (21.42%) were >1, which indicates a positive selection on these paralogs. The CaABCF8-CaABCF2 pair had the highest ω ratios with 1.57.

The duplication time of *Capsicum* ABC paralog pairs was estimated by using a relative Ks measure as a proxy for time, and it spanned from 1 to 84 million years ago (MYA), with an average duplication time of ~26 MYA. Multiple copies of genes in a gene family could have evolved due to the flexibility provided by events of whole-genome tandem and segmental duplications. Gene duplication, segmental or tandem, has been documented in several plant gene families, such as NAC, MYB, F-box, bZIP and ABC transporters [20, 61]. The ω ratios for 3 pairs of paralogs were > 1, representing positive selection and fast evolutionary rates in these ABC paralogs at the protein level. This finding differs from other gene families in plants, such as BURP in Medicago and ACD in tomato, which contain a few or even no paralog pairs undergoing positive selection [62, 63]. In our study, a relatively large percentage (~ 21%) of ABC paralogs pairs underwent positive selection. We assumed that these paralog gene pairs might have evolved in order to acquire new functions and adjust to their living environment.

Table 2. Ka-Ks calculation of each pair of syntenic Capsicum ABC paralogs.

Syntenic paralog pairs	S-Sites	N-Sites	Ka	Ks	Ka/Ks	Selection pressure	Duplication time (MYA)
CbABCG11-CbABCG40	127.23	406.77	0.23	0.21	1.06	Positive selection	15.36
CaABCG2-CcABCG40	142.31	490.69	0.23	0.25	0.89	Purifying selection	18.13
CaABCF8-CaABCF2	91.15	310.85	0.07	0.05	1.57	Positive selection	3.25
CcABCC5-CcABCC22	88.18	256.82	0.11	0.15	0.72	Purifying selection	11.12
CbABCB36-CaABCB9	260.28	843.72	0.00	0.02	0.06	Purifying selection	1.40
CaABCB31-CcABCB3	326.66	996.34	0.07	0.08	0.82	Purifying selection	6.00
CaABCG3-CaABCG34	49.75	166.25	0.10	0.09	1.13	Positive selection	6.11
CaABCG30-CaABCG80	108.39	371.61	0.52	0.54	0.95	Purifying selection	39.06
CbABCG89-CcABCG69	497.18	1704.82	0.02	0.08	0.31	Purifying selection	5.47
CaABCG23-CaABCG33	401.44	1314.56	0.01	0.01	0.47	Purifying selection	0.99
CbABCG7-CcABCG38	84.41	281.59	0.17	0.20	0.84	Purifying selection	14.57
CbABCG72-CbABCG73	52.13	184.87	0.82	1.00	0.82	Purifying selection	72.07
CbABCG29-CbABCG30	95.05	327.95	0.96	1.16	0.83	Purifying selection	83.55
CbABCG58-CaABCG38	148.66	514.34	1.01	1.15	0.88	Purifying selection	82.39

S-Sites, number of synonymous sites; N-Sites, number of non-synonymous sites; Ka, non-synonymous substitution rate; Ks, synonymous substitution; MYA, million years ago.

https://doi.org/10.1371/journal.pone.0215901.t002

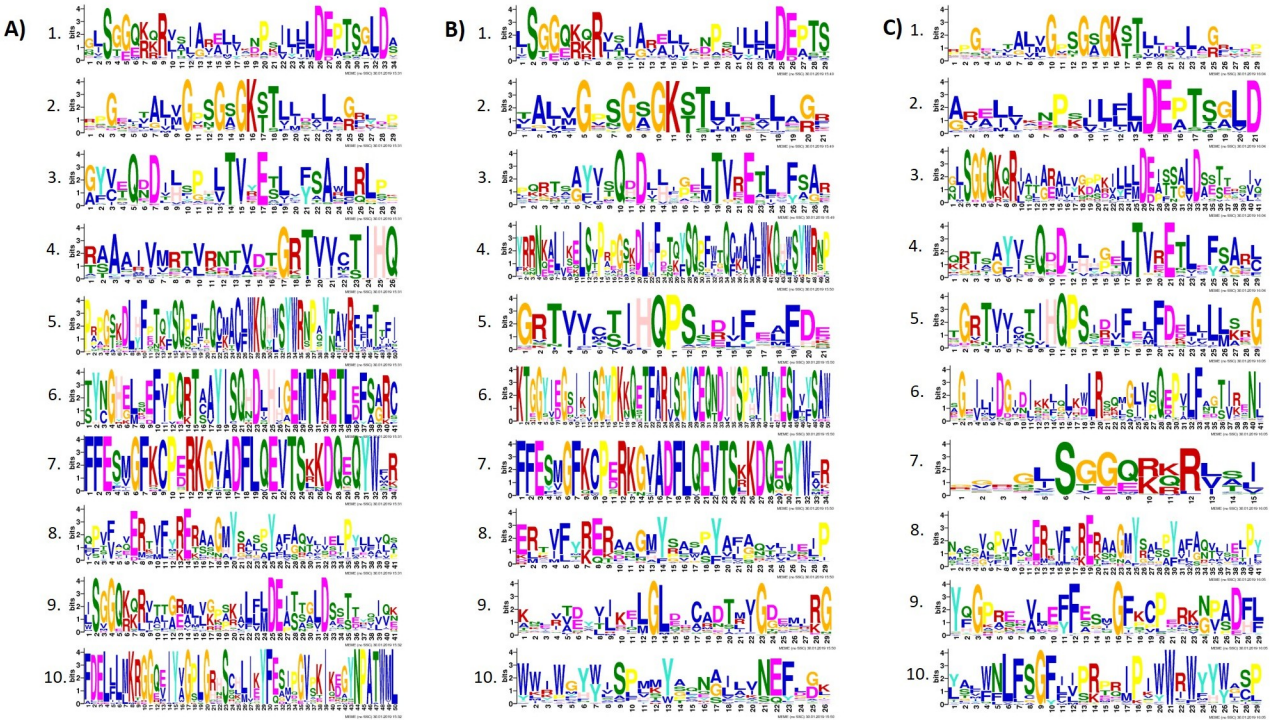

Fig 5. Conserved motifs of ABC transporter proteins in Capsicum species. (A) *C. annuum*, (B) *C. baccatum* and (C) *C. chinense*.

https://doi.org/10.1371/journal.pone.0215901.g005

Expression correlation analysis of syntenic ABC paralog pairs across different tissues and under stress treatments could help to reveal their functional roles in evolutionary fates.

Motif composition and *Cis*-elements of *Capsicum* ABC genes

MEME analysis according to domain composition of pepper ABC transporter proteins revealed 10 conserved motifs in ABCA-G and ABCI families (Fig 5 and S4 Table). The lengths of the conserved motifs ranged from 15 to 50 amino acids. Additionally, the number of conserved motifs in each *Capsicum* ABC transporters ranged from 1 to 8. The information obtained from ScanProsite analysis revealed that the function of most of the motifs was pleiotropic drug resistance related to the ABCG subfamily. All conserved motifs predicted have similar properties as ABC transporters, and the signature motif (LSGGQ) was found in most of the *Capsicum* ABC transporters.

In order to identify putative *cis*-elements in the *Capsicum* ABC promoters, 1500 bp DNA sequences upstream of the start codon (ATG) for the ABC transporters for each species were analyzed by using the Plant *Cis*-acting Regulatory DNA Elements (PLACE) website. The analysis identified 124 different *cis*-elements in all *Capsicum* ABC transporters. A total of 23 common *cis*-regulatory elements were present across all the promoter regions of the ABC transporters and were highly conserved among all *Capsicum* species (Table 3).

Four common cis-regulatory elements, CATATGGMSAUR, ASF1MOTIFCAMV, NTBBF1 ARROLB and ARFAT, were found related to plant hormones including auxin, auxin response factor (ARF) and Small Auxin-Up RNAs (SAUR), which suggests that these plant hormones could affect the expression of *Capsicum* ABC transporters and can affect the plant growth and development. The WRKY71OS *cis*-regulatory element is responsive to stresses caused by pathogens. Out of the 23-common *cis*-regulatory elements, TBOXATGAPB, BOXIIPCCHS, INRNTPSADB, and GT1CONSENSUS are thought to be required for transcriptional

Table 3. Common putative *cis*-elements identified in the promoter sequences of ABC proteins genes in Capsicum species.

Cis-element	Signal sequence	SITE	Expression patern
ABRELATERD1	ACGTG	S000414	ABRE, etiolation, erd
GCN4OSGLUB1	TGAGTCA	S000277	GluB-1, glutelin, endosperm, seed, storage protein, GCN4 motif
TATABOX4	TATATAA	S000111	TATA, sporamin, phaseolin
CATATGGMSAUR	CATATG	S000370	SAUR, NDE, auxin
ASF1MOTIFCAMV	TGACG	S000024	TGACG, root, leaf, CaMV, 35S, promoter, auxin, salicylic acid
NTBBF1ARROLB	ACTTTA	S000273	rolB, Dof, auxin, domain B, root, shoot, meristem, vascular
ARFAT	TGTCTC	S000270	auxin, AuxRE, ARF, ARF1, Aux/IAA, SAUR, NDE, GH3, D1, D4
CAATBOX1	CAAT	S000028	CAAT, legA, seed
CCAATBOX1	CCAAT	S000030	HSE (Heat shock element), CCAAT box
HEXMOTIFTAH3H4	ACGTCA	S000053	hexamer, HBP-1A, HBP-1B, histone H3, CaMV, 35S, NOS, HBP-1
T/GBOXATPIN2	AACGTG	S000458	T/G-box, JA, pin2, LAP, MYC, wounding
TATCCAYMOTIFOSRAMY3D	TATCCAY	S000256	GATA, amylase, sugar, repression
LTRE1HVBLT49	CCGAAA	S000250	low temperature, LTRE
GT1CONSENSUS	GRWAAW	S000198	GT-1, light, TATA, TFIIA, TBP, HR, SAR, TMV, leaf, shoot
INRNTPSADB	YTCANTYY	S000395	initiater, light-responsive transcription, TATA-less promoter
TATCCAOSAMY	TATCCA	S000403	alpha-amylase, MYB proteins, gibberellin, GA, sugar starvation
TGACGTVMAMY	TGACGT	S000377	alpha-Amylase, cotyledon, seed germination, seed
ABREATCONSENSUS	YACGTGGC	S000406	ABA, ABF, bZIP factors
BOXIIPCCHS	ACGTGGC	S000229	Box II, Box 2, CHS, chs, light regulation
LRENPCABE	ACGTGGCA	S000231	CAB, cab, cab-E, CABE, light, leaf, shoot
WRKY71OS	TGAC	S000447	WRKY, GA, MYB, W box, TGAC, PR proteins
LTRECOREATCOR15	CCGAC	S000153	low temperature, cold, LTRE, drought, ABA, cor15a, BN115, leaf
TBOXATGAPB	ACTTTG	S000383	GAPB, glyceraldehyde-3-phosphate dehydrogenase, light-activated

https://doi.org/10.1371/journal.pone.0215901.t003

regulation by light. Two common *cis*-elements, CCAATBOX1 and LTRECOREATCOR15 were identified to response to low temperature, cold, drought and heat shock, which suggests that *Capsicum* ABC transporters might be involved in response to abiotic stress.

GO annotation of ABC transporter genes

GO analysis performed with Blast2Go suggested the putative participation of ABC genes in multiple biological processes, molecular functions, and cellular component (Fig 6). GO results

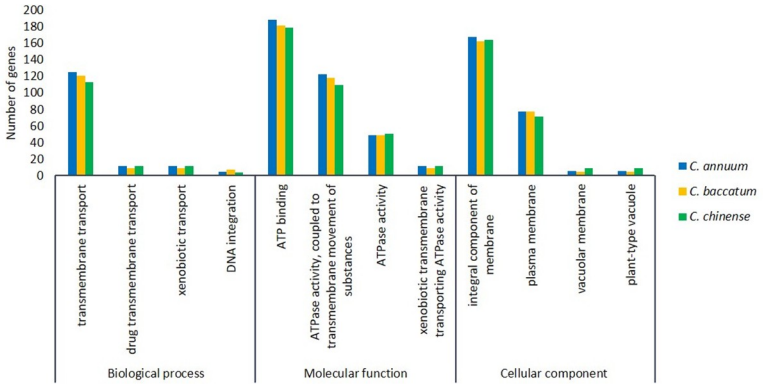

Fig 6. Detailed gene ontology analysis results for *Capsicum* species. Biological process, cellular component, and molecular function were identified with the Blast2GO program.

https://doi.org/10.1371/journal.pone.0215901.g006

indicated the putative participation of *Capsicum* ABC transporters in transmembrane transport as a principal biological process, as well as drug transmembrane transport, xenobiotic transport and DNA integration. ATP binding and ATPase activity coupled to transmembrane of substances were the main activities for molecular function. Most of the ABC transporters were classified in the integral component of the membrane for cellular localization followed by the plasma membrane. In all species, 18 ABC transporters from *C. chinense*, 12 from *C. annuum* and 12 from *C. baccatum* were cellular localized in the vacuolar membrane and plant-type vacuole. In pepper fruit, capsaicinoids are synthesized exclusively in placental tissue and accumulate in vacuoles of placental epidermal cells [64], so ABC transporters might participate in vacuolar capsaicinoid uptake and transport, affecting the capsaicinoid content in pepper fruits.

Capsaicinoid levels are highly dynamic during fruit development. Their levels appear to be influenced by the ontogenetic trajectory of the fruit. Capsaicinoids begin to accumulate from the early stages (10 dpa) of fruit development, peak at about 40 dpa, and then it decreases sharply [65]. The late decrease in capsaicinoid content appears to result from high peroxidase activity, which oxidizes capsaicinoids in the presence of hydrogen peroxide (H_2O_2) [66, 67]. A gene CcABCC12 from *C. chinense* was found to have a H_2O_2 catabolic process as a biological process resulting in the breakdown of H_2O_2 (S5 Table), which suggests a detoxification process of H_2O_2 exclusively for *C. chinense* and a subsequent high content of capsaicinoids. Another factor that can affects the metabolism of capsaicinoids is mineral nutrition. Nitrogen (N) and potassium (K) are the main mineral players. Nitrogen availability in soil directly affects capsaicin accumulation since a single capsaicin molecule synthesis involves three amino acids such as phenylalanine, valine and leucine [68]. By contrast, potassium does not participate in capsaicinoid metabolism, however it has been reported that an increase in potassium concentration significantly decreases the capsaicin levels and leaf nitrogen content in *C. chinense* [69]. Thus, the level of potassium might indirectly affect capsaicin accumulation via its effects on fruit development [70]. CcABCC1 in *C. chinense* showed cellular potassium ion homeostasis as a biological process. The principal function of this biological process involves maintenance of an internal steady state of potassium ions at the level of a cell. In fact, *C. chinense* was found to have the highest values for capsaicin and dihydrocapsaicin, suggesting that cellular potassium ion homeostasis may indirectly affect capsaicinoid levels in pepper fruits.

Expression profile of ABC transporters in *C. annuum* and *C. chinense*

A BLASTN strategy was used to identify the orthologs for Capsaicinoid markers previously identified by [5] for the CA06g14430 gene from the SGN database. The resulted orthologs were CaABCG28, CbABCG26, and CcABCG37 corresponding to each of the species. For CA11g09150, the orthologs were CaABCC9, CBABCC5 and CcABCC20. The main purpose of gene expression profiling is to determine the genes that are differentially expressed within the organism being studied. In the same way, we used a BLASTN search to identify the orthologs between *C. annuum* and *C. chinense* to correlate their expression in placental tissues. In order to characterize the expression patterns of individual *Capsicum* ABC transporters at different stages (6, 16 and 25 dpa), we used publicly available RNA-seq data for *C. annuum* cv. CM334 [2]. The RPKM values for green fruits (16 dpa) from two varieties of *C. chinense* (Naga morich and Pimienta de neyde) and *C. annuum* cv CM334 were plotted in a hierarchical heatmap (Fig 7).

The *C. annuum* CM334 variety showed a similar pattern of expression in all placenta tissue stages (Fig 7A). CaABCG11 was expressed at 6 and 16 dpa with a higher expression at 6 dpa. On the other hand, CaABCB36, CaABCG83 and CaABCG87 were highly expressed at 16 dpa.

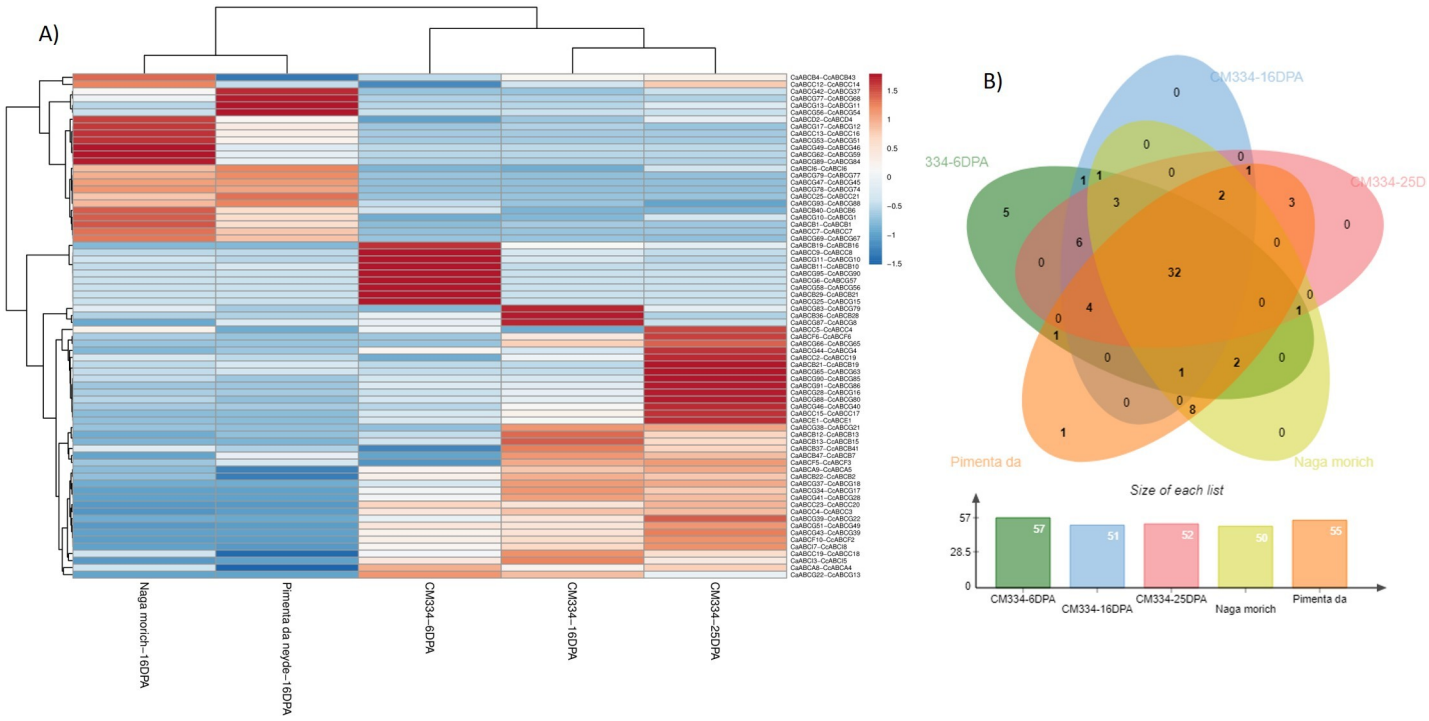

Fig 7. Expression patterns of ABCs transporters in placenta tissue of C. annuum var CM344 and C. chinense. A) Heat map of expression profiles (in log2-based RPKM) from placenta tissues (6, 16, 35 days post-anthesis (dpa)) of *C. annuum* and placenta tissues at 16 dpa of two *C. chinense* varieties (Naga morich and Pimienta da neyde). The expression levels are represented by the color bar: red, upregulated, and blue, downregulated. B) Venn diagram analysis of the tissue expression of CaABC (C. annuum) -CcABC (C. chinense) transporters.

https://doi.org/10.1371/journal.pone.0215901.g007

Most *Capsicum* ABC transporters presented different expression patterns, whereas a few resulted similar. Some exhibited stage- and species-specific expression, which suggests that these genes may play specific roles in the relevant stages and *Capsicum* species. Among 74 genes, 32 were expressed across all placenta tissues at different stages (Fig 7B). The ABC transporters previously described as a major marker for capsaicin and dihydrocapsaicin content were mostly expressed in *C. annuum* cv. CM334. CaABCC9 and CcABCC20 (CA11g01950) were found greatly expressed at 16 and 25 dpa and CaABCG28 (CA06g14430) was found in 25-dpa tissue. By contrast, CcABCG37 (CA06g14430) was greatly expressed only *C. chinense* cv. Naga morich at 16 dpa. Mainly, the ABCC and ABCG subfamilies were distributed across different stages; however, only ABCA, ABCB, ABCE, ABCF and ABCI members were expressed in *C. annuum* cv. CM334, and ABCD members were expressed in *C. chinense* cultivars. *C. chinense* varieties at 16 dpa shared the expression of eight genes (CcABCC16, CcABCC21, CcABCG45, CcABCG46, CcABCG51, CcABCG68, CcABCG74, CcABCG84). CcABCG54 was exclusively expressed in Pimienta de neyde, whereas CcABCG12, CcABCG16, CcABCG46, CcABCG51, CcABCG59, CcABCG84 and CcABCD4 were highly expressed in Naga morich. Most of the genes expressed in the *C. chinense* varieties belonged to the ABCC, ABCG and ABCD families.

The ABCA subfamily is not yet fully functionally characterized in plants; it has been reported to be related to pollen and seed germination and maturation [71]. The presence of one full-sized ABCA transporter was exclusive to dicots, including pepper, *Arabidopsis* [13], tomato [59], *B. rapa* [20], and *B. napus* [21] but so far it has not been identified in monocots, such as rice [16] and maize [18]. However, Chen et al. [58] reported one full-sized ABCA

transporter in pineapple. The ABCB subfamily is composed of a full-sized or multidrug resistance (MDR) protein and half-sized protein, with names such as transporters associated with antigen processing (TAP) and ABC transporter of mitochondria (ATM) [10]. In plants, ABCB is the second largest subfamily. For instance, in *Arabidopsis*, the ABCB subfamily participates in different processes such as auxin bidirectional transport, phospholipid translocation, stomatal regulation, berberine transport, Fe/S biogenesis and metal stress (Cd and Al) tolerance [72]. AtABCB1, a member of AtABCB, has been proposed to participate in auxin transportation, and AtABCB1-overexpressing plants show long hypocotyls [73, 74].

ABCE family members are soluble ABC proteins and are also called RNase L inhibitor (RLI). They possess an N-terminal Fe-S domain, which interacts with nucleic acids [75]. Their main function have been reported to be related to control of translation and ribosome biogenesis [76]. Similarly, ABCE and ABCF family members are soluble proteins and contain an NBD-NBD domain structure. In *Arabidopsis*, ABCF (AtABCF3) proteins have been reported to play a role in root growth [77].

The ABCG subfamily, also called pleiotropic drug resistance or white–brown complex proteins, is the largest subfamily in plants. It has been reported that ABCGs transport various phytohormones, including abscisic acid, cytokinin, strigolactone and auxin derivatives [78]. The subcellular localization of full-sized ABCGs is the plasma membrane [79], whereas half-sized ABCGs are complex proteins and have been localized in the plasma membrane, mitochondrial membrane, chloroplast membrane and cytoplasm [18]. Full-sized ABCGs of *Arabidopsis*, AtABCG32 [80], and rice OsABCG31 [81] are involved principally in cuticle formation, while half-sized ABCGs play an important physiological role like cuticle formation, kanamycin resistance, abscisic acid exporter and pollen development [82–84]. In cotton, GhWBC1 a half-sized white-brown complex member has been reported to be involved in fiber cell elongation [85]. Shibata et al. [86] reported that ABCG subfamily may play a key role in export of the antimicrobial diterpene such as sesquiterpenoid phytoalexin and capsidiol for resistance to the potato late blight pathogen *Phytophthora infestans* in *Nicotiana benthamiana*.

The ABCC subfamily is also called MDR-associated proteins (MRP) because of their function in transporting glutathione- and glucuronide-conjugates in drug resistance (Verrier et al., 2008). Pang et al. [18] reported that most plant ABCCs are characterized as vacuolar localized proteins and a few of them have been reported to reside on the plasma membrane. The function of different ABCC members has been found in diverse plants; for example, *Arabidopsis* AtABCC5 [87], maize ZmMRP4 [88] and rice OsABCC13 [89] are implicated in phytate transport. AtABCC1 and AtABCC4 are involved in folate transport, while maize ZmMRP3 and grape VvABCC1 play an important role in anthocyanin accumulation in vacuoles [90, 91]. The high expression of the ABCC subfamily in pepper placental tissue of two species, and its principal function reported in other plants, suggest that the ABCC subfamily function in *Capsicum* spp. is the transport and accumulation of capsaicin in vacuoles of the placental tissue.

Gene expression analysis

We selected CaABCG28, CbABCG26 and CcABCG37 (orthologs of CA06g14430); as well as CaABCC9, CBABCC5 and CcABCC20 (orthologs of CA11g09150) for gene expression analysis by RT-qPCR (Fig 8). Gene expression was detected throughout all placenta stages and species analyzed. The orthologs of CA06g14430 corresponding to the ABCG family showed a similar expression pattern in the *C. chinense* varieties at different stages, but at 16 dpa, higher relative expression was found for the Naga morich variety and the lowest was for *C. annuum* cv. CM334 (Fig 8A). At 6 dpa, the expression was similar across all cultivars, but it was less in *C. baccatum* and *C. chinense* cv. Pimienta de neyde at 16 dpa. The remaining varieties showed

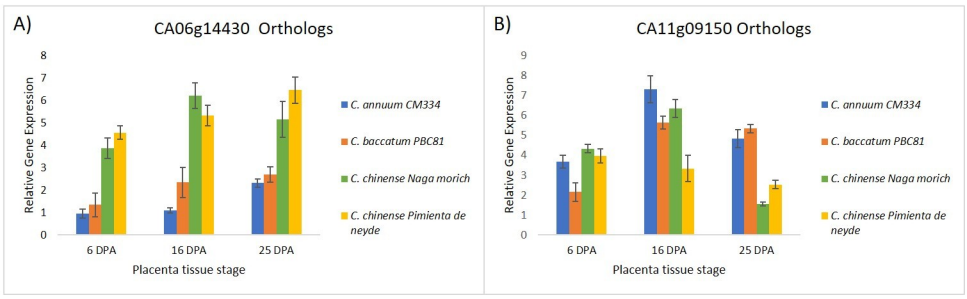

Fig 8. Gene expression analysis of selected ABC transporters in placenta tissues at 6, 16 and 25 days post-anthesis (dpa) across Capsicum species. RT-qPCR analysis for A) CA06g14430 homologs (CaABCG28, CbABCG26, and CcABCG37) and B) CA11g09150 orthologs (CaABCC9, CBABCC5 and CcABCC20). Values are means ± SD; n = 3.

https://doi.org/10.1371/journal.pone.0215901.g008

an expression pattern close to that of the ABCC family (Fig 8B). At 25 dpa, the highest expression was found for *C. annuum* cv. CM334, followed by *C. baccatum* cv. PBC81 and the lowest for the *C. chinense* varieties. Different patterns in the expression between the orthologs of the Capsaicinoids markers previously mentioned suggest that the expression may be specie-specific for each of the ABC subfamilies in *Capsicum* species.

Conclusion

Although the ABC transporter gene superfamily has been widely studied among extant organisms including plants, the present study is the first to report the presence of 572 putative ABC transporter proteins in the entire pepper genome sequences of three different *Capsicum* species. Our results provide fundamental and exhaustive information about the pepper ABC transporters by performing a comprehensive genome-wide identification and expression patterns of these proteins family. Based on their evolutionary origin, phylogenetic analysis classified the ABC proteins into 8 main subfamilies (designated A to G, and I). Chromosomal mapping revealed that members of ABCG, ABCB and ABCC subfamilies were the most abundant genes, whereas the ABCD and ABCE subfamilies were manifested in a lesser abundance. Our results suggest that the ABC transporters, specifically the ABCC and ABCG subfamilies, interfere in capsaicin and dihydrocapsaicin content in pepper. Indirectly, these two subfamilies may be involved in the transportation of secondary metabolites such as capsaicinoids to the placenta vacuoles for their storage. Moreover, we suggest that the ABBC and ABCG subfamilies play a role in the H_2O_2 detoxification process to reduce capsaicin degradation, specifically in the *C. chinense* fruits. Our study will provide clues for further research on the evolution of the ABC transporter gene family and their influence in specific biological functions of *Capsicum* fruits including plant growth, development and capsaicinoid content in pepper.

Supporting information

S1 Table. Basic information on the ABC transporter gene family in *C. annuum*.
(XLSX)

S2 Table. Basic information on the ABC transporter gene family in *C. baccatum*.
(XLSX)

S3 Table. Basic information on the ABC transporter gene family in *C. chinense*.
(XLSX)

S4 Table. Conserved motifs present in ABC transporter in *Capsicum* species.
(XLSX)

S5 Table. Detailed information on gene ontology annotation results for *Capsicum* species.
(XLSX)

S6 Table. RPKM values for placenta tissues of orthologs of ABC transporter pairs of *C. annuum* and *C. chinense*.
(XLSX)

Acknowledgments

Authors acknowledge funding from USDA-NIFA grant 2016–06616 and the Department of Biotechnology, New Delhi, India for the associateship extended to Sudip Kumar Dutta, Scientist (Horticulture), ICAR RC NEH Region, Mizoram Centre, Kolasib, Mizoram-796081, India.

Author Contributions

Conceptualization: Carlos Lopez-Ortiz, Padma Nimmakayala, Umesh K. Reddy.

Data curation: Carlos Lopez-Ortiz, Purushothaman Natarajan.

Formal analysis: Carlos Lopez-Ortiz, Sudip Kumar Dutta, Purushothaman Natarajan, Yadira Peña-Garcia, Umesh K. Reddy.

Funding acquisition: Padma Nimmakayala, Umesh K. Reddy.

Investigation: Umesh K. Reddy.

Methodology: Carlos Lopez-Ortiz, Sudip Kumar Dutta, Purushothaman Natarajan, Yadira Peña-Garcia, Venkata Abburi, Thangasamy Saminathan, Umesh K. Reddy.

Project administration: Padma Nimmakayala, Umesh K. Reddy.

Resources: Padma Nimmakayala, Umesh K. Reddy.

Software: Carlos Lopez-Ortiz, Yadira Peña-Garcia, Umesh K. Reddy.

Supervision: Carlos Lopez-Ortiz, Padma Nimmakayala, Umesh K. Reddy.

Validation: Carlos Lopez-Ortiz.

Visualization: Padma Nimmakayala, Umesh K. Reddy.

Writing – original draft: Carlos Lopez-Ortiz, Sudip Kumar Dutta, Yadira Peña-Garcia, Umesh K. Reddy.

Writing – review & editing: Carlos Lopez-Ortiz, Yadira Peña-Garcia, Padma Nimmakayala, Umesh K. Reddy.

References

1. Ferrari C, Torres EAFdS. Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. Biomedicine & Pharmacotherapy. 2003; 57(5–6):251–60.
2. Kim S, Park M, Yeom S-I, Kim Y-M, Lee JM, Lee H-A, et al. Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. Nature genetics. 2014; 46(3):270. https://doi.org/10.1038/ng.2877 PMID: 24441736
3. Qin C, Yu C, Shen Y, Fang X, Chen L, Min J, et al. Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. Proceedings of the National Academy of Sciences. 2014; 111(14):5135–40.

4. Perla V, Nadimi M, Reddy R, Hankins GR, Nimmakayala P, Harris RT, et al. Effect of ghost pepper on cell proliferation, apoptosis, senescence and global proteomic profile in human renal adenocarcinoma cells. PloS one. 2018; 13(10):e0206183. https://doi.org/10.1371/journal.pone.0206183 PMID: 30379886
5. Nimmakayala P, Abburi VL, Saminathan T, Alaparthi SB, Almeida A, Davenport B, et al. Genome-wide diversity and association mapping for capsaicinoids and fruit weight in *Capsicum annuum* L. Scientific reports. 2016; 6:38081. https://doi.org/10.1038/srep38081 PMID: 27901114
6. Dassa E, Bouige P. The ABC of ABCs: a phylogenetic and functional classification of ABC systems in living organisms. Research in microbiology. 2001; 152(3–4):211–29. PMID: 11421270
7. Jones P, George A. The ABC transporter structure and mechanism: perspectives on recent research. Cellular and Molecular Life Sciences CMLS. 2004; 61(6):682–99. https://doi.org/10.1007/s00018-003-3336-9 PMID: 15052411
8. Higgins CF. ABC transporters: from microorganisms to man. Annual review of cell biology. 1992; 8 (1):67–113.
9. Rea PA. Plant ATP-binding cassette transporters. Annu Rev Plant Biol. 2007; 58:347–75. https://doi.org/10.1146/annurev.arplant.57.032905.105406 PMID: 17263663
10. Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, et al. Plant ABC proteins–a unified nomenclature and updated inventory. Trends in plant science. 2008; 13(4):151–9. https://doi.org/10.1016/j.tplants.2008.02.001 PMID: 18299247
11. Davies T, Coleman J. The *Arabidopsis thaliana* ATP-binding cassette proteins: an emerging superfamily. Plant, Cell & Environment. 2000; 23(5):431–43.
12. Wang B, Dukarevich M, Sun EI, Yen MR, Saier MH. Membrane porters of ATP-binding cassette transport systems are polyphyletic. Journal of Membrane Biology. 2009; 231(1):1. https://doi.org/10.1007/s00232-009-9200-6 PMID: 19806386
13. Ro Sánchez-Fernández, Davies TE Coleman JO, Rea PA. The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory. Journal of Biological Chemistry. 2001; 276(32):30231–44. https://doi.org/10.1074/jbc.M103104200 PMID: 11346655
14. Martinoia E, Klein M, Geisler M, Bovet L, Forestier C, Kolukisaoglu Ü, et al. Multifunctionality of plant ABC transporters–more than just detoxifiers. Planta. 2002; 214(3):345–55. PMID: 11855639
15. Moon S, Jung K-H. Genome-wide expression analysis of rice ABC transporter family across spatio-temporal samples and in response to abiotic stresses. Journal of plant physiology. 2014; 171(14):1276–88. https://doi.org/10.1016/j.jplph.2014.05.006 PMID: 25014263
16. Saha J, Sengupta A, Gupta K, Gupta B. 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. 2015; 54:18–32. https://doi.org/10.1016/j.compbiolchem.2014.11.005 PMID: 25531538
17. Lane TS, Rempe CS, Davitt J, Staton ME, Peng Y, Soltis DE, et al. Diversity of ABC transporter genes across the plant kingdom and their potential utility in biotechnology. BMC biotechnology. 2016; 16 (1):47. https://doi.org/10.1186/s12896-016-0277-6 PMID: 27245738
18. 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(2):411–28. https://doi.org/10.1016/j.gene.2013.05.051 PMID: 23747399
19. Chen L, Li Y-x, Li C, Shi Y, Song Y, Zhang D, et al. Genome-wide analysis of the pentatricopeptide repeat gene family in different maize genomes and its important role in kernel development. BMC plant biology. 2018; 18(1):366. https://doi.org/10.1186/s12870-018-1572-2 PMID: 30567489
20. Yan C, Duan W, Lyu S, Li Y, Hou X. Genome-wide identification, evolution, and expression analysis of the ATP-binding cassette transporter gene family in *Brassica rapa*. Frontiers in plant science. 2017; 8:349. https://doi.org/10.3389/fpls.2017.00349 PMID: 28367152
21. Zhang XD, Zhao KX, Yang ZM. Identification of genomic ATP binding cassette (ABC) transporter genes and Cd-responsive ABCs in *Brassica napus*. Gene. 2018; 664:139–51. https://doi.org/10.1016/j.gene.2018.04.060 PMID: 29709635
22. Çakır B, Kılıçkaya O. Whole-genome survey of the putative ATP-binding cassette transporter family genes in *Vitis vinifera*. PloS one. 2013; 8(11):e78860. https://doi.org/10.1371/journal.pone.0078860 PMID: 24244377
23. Theodoulou FL, Holdsworth M, Baker A. Peroxisomal ABC transporters. Febs Letters. 2006; 580 (4):1139–55. https://doi.org/10.1016/j.febslet.2005.12.095 PMID: 16413537
24. Ito H, Gray WM. A gain-of-function mutation in the *Arabidopsis* pleiotropic drug resistance transporter PDR9 confers resistance to auxinic herbicides. Plant physiology. 2006; 142(1):63–74. https://doi.org/10.1104/pp.106.084533 PMID: 16877699

25. Kang J, Park J, Choi H, Burla B, Kretzschmar T, Lee Y, et al. Plant ABC transporters. The *Arabidopsis* book/American Society of Plant Biologists. 2011; 9.
26. Jing H, Li C, Ma F, Ma J-H, Khan A, Wang X, et al. Genome-wide identification, expression diversication of dehydrin gene family and characterization of CaDHN3 in pepper (*Capsicum annuum* L.). PloS one. 2016; 11(8):e0161073. https://doi.org/10.1371/journal.pone.0161073 PMID: 27551973
27. ZHANG H, Ning C, Chunjuan D, SHANG Q. Genome-wide Identification and Expression of ARF Gene Family during Adventitious Root Development in Hot Pepper (*Capsicum annuum*). Horticultural plant journal. 2017; 3(4):151–64.
28. Khan A, Li R-J, Sun J-T, Ma F, Zhang H-X, Jin J-H, et al. Genome-wide analysis of dirigent gene family in pepper (*Capsicum annuum* L.) and characterization of CaDIR7 in biotic and abiotic stresses. Scientific reports. 2018; 8(1):5500. https://doi.org/10.1038/s41598-018-23761-0 PMID: 29615685
29. Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, et al. Phytozome: a comparative platform for green plant genomics. Nucleic acids research. 2011; 40(D1):D1178–D86.
30. Eddy SR. Profile hidden Markov models. Bioinformatics (Oxford, England). 1998; 14(9):755–63.
31. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, et al. Pfam: the protein families database. Nucleic acids research. 2013; 42(D1):D222–D30.
32. Letunic I, Bork P. 20 years of the SMART protein domain annotation resource. Nucleic acids research. 2017; 46(D1):D493–D6.
33. Potter SC, Luciani A, Eddy SR, Park Y, Lopez R, Finn RD. HMMER web server: 2018 update. Nucleic acids research. 2018; 46(W1):W200–W4. https://doi.org/10.1093/nar/gky448 PMID: 29905871
34. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution. 2018; 35(6):1547–9. https://doi.org/10.1093/molbev/msy096 PMID: 29722887
35. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research. 1997; 25(24):4876–82. PMID: 9396791
36. Letunic I, Bork P. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics. 2006; 23(1):127–8. https://doi.org/10.1093/bioinformatics/btl529 PMID: 17050570
37. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic acids research. 2003; 31(13):3784–8. PMID: 12824418
38. Higo K, Ugawa Y, Iwamoto M, Korenaga T. Plant cis-acting regulatory DNA elements (PLACE) database: 1999. Nucleic acids research. 1999; 27(1):297–300. PMID: 9847208
39. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, et al. MEME SUITE: tools for motif discovery and searching. Nucleic acids research. 2009; 37(suppl_2):W202–W8.
40. De Castro E, Sigrist CJ, Gattiker A, Bulliard V, Langendijk-Genevaux PS, Gasteiger E, et al. ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. Nucleic acids research. 2006; 34(suppl_2):W362–W5.
41. Gu X, Wang Y, Gu J. Age distribution of human gene families shows significant roles of both large-and small-scale duplications in vertebrate evolution. Nature genetics. 2002; 31(2):205. https://doi.org/10.1038/ng902 PMID: 12032571
42. Wang D, Zhang Y, Zhang Z, Zhu J, Yu J. KaKs_Calculator 2.0: a toolkit incorporating gamma-series methods and sliding window strategies. Genomics, proteomics & bioinformatics. 2010; 8(1):77–80.
43. Moniz de Sa M, Drouin G. Phylogeny and substitution rates of angiosperm actin genes. Molecular biology and evolution. 1996; 13(9):1198–212. https://doi.org/10.1093/oxfordjournals.molbev.a025685 PMID: 8896372
44. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet journal. 2011; 17(1):10–2.
45. Joshi N, Fass J. Sickle-A windowed adaptive trimming tool for FASTQ files using quality. Online publication https://githubcom/najoshi/sickle[Google Scholar]. 2011.
46. Li H, Durbin R. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics. 2010; 26(5):589–95. https://doi.org/10.1093/bioinformatics/btp698 PMID: 20080505
47. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics. 2015; 31(2):166–9. https://doi.org/10.1093/bioinformatics/btu638 PMID: 25260700
48. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic acids research. 2015; 43(W1):W566–W70. https://doi.org/10.1093/nar/gkv468 PMID: 25969447

49. Fernandez-Pozo N, Menda N, Edwards JD, Saha S, Tecle IY, Strickler SR, et al. The Sol Genomics Network (SGN)—from genotype to phenotype to breeding. Nucleic acids research. 2014; 43(D1): D1036–D41.
50. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001; 25(4):402–8. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609
51. Wan H, Yuan W, Ruan M, Ye Q, Wang R, Li Z, et al. Identification of reference genes for reverse transcription quantitative real-time PCR normalization in pepper (*Capsicum annuum* L.). Biochemical and biophysical research communications. 2011; 416(1–2):24–30. https://doi.org/10.1016/j.bbrc.2011.10.105 PMID: 22086175
52. Hoffman PG, Lego MC, Galetto WG. Separation and quantitation of red pepper major heat principles by reverse-phase high-pressure liquid chromatography. Journal of Agricultural and Food Chemistry. 1983; 31(6):1326–30.
53. Govindarajan V, Sathyanarayana M. *Capsicum*—production, technology, chemistry, and quality. Part V. Impact on physiology, pharmacology, nutrition, and metabolism; structure, pungency, pain, and desensitization sequences. Critical Reviews in Food Science & Nutrition. 1991; 29(6):435–74.
54. Sanatombi K, Sharma G. In vitro propagation of Capsicum chinense Jacq. Biologia Plantarum. 2008; 52(3):517–20.
55. Garcés-Claver A, Fellman SM, Gil-Ortega R, Jahn M, Arnedo-Andrés MS. Identification, validation and survey of a single nucleotide polymorphism (SNP) associated with pungency in Capsicum spp. Theoretical and Applied Genetics. 2007; 115(7):907–16. https://doi.org/10.1007/s00122-007-0617-y PMID: 17882396
56. Manivannan A, Kim J-H, Yang E-Y, Ahn Y-K, Lee E-S, Choi S, et al. Next-Generation Sequencing Approaches in Genome-Wide Discovery of Single Nucleotide Polymorphism Markers Associated with Pungency and Disease Resistance in Pepper. BioMed research international. 2018;2018.
57. Liu S, Li W, Wu Y, Chen C, Lei J. De novo transcriptome assembly in chili pepper (*Capsicum frutescens*) to identify genes involved in the biosynthesis of capsaicinoids. PloS one. 2013; 8(1):e48156. https://doi.org/10.1371/journal.pone.0048156 PMID: 23349661
58. 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* (L.) Merr.) reveal the role of AcABCG38 in pollen development. Frontiers in plant science. 2017; 8:2150. https://doi.org/10.3389/fpls.2017.02150 PMID: 29312399
59. Ofori PA, Mizuno A, Suzuki M, Martinoia E, Reuscher S, Aoki K, et al. Genome-wide analysis of ATP binding cassette (ABC) transporters in tomato. PloS one. 2018; 13(7):e0200854. https://doi.org/10.1371/journal.pone.0200854 PMID: 30048467
60. Linton KJ, Higgins CF. Structure and function of ABC transporters: the ATP switch provides flexible control. Pflügers Archiv-European Journal of Physiology. 2007; 453(5):555–67. https://doi.org/10.1007/s00424-006-0126-x PMID: 16937116
61. Baloglu MC, Eldem V, Hajyzadeh M, Unver T. Genome-wide analysis of the bZIP transcription factors in cucumber. PloS one. 2014; 9(4):e96014. https://doi.org/10.1371/journal.pone.0096014 PMID: 24760072
62. Li Y, Chen X, Chen Z, Cai R, Zhang H, Xiang Y. Identification and expression analysis of BURP domain-containing genes in *Medicago truncatula*. Frontiers in plant science. 2016; 7:485. https://doi.org/10.3389/fpls.2016.00485 PMID: 27148311
63. Paul A, Rao S, Mathur S. The α-crystallin domain containing genes: identification, phylogeny and expression profiling in abiotic stress, phytohormone response and development in tomato (*Solanum lycopersicum*). Frontiers in plant science. 2016; 7:426. https://doi.org/10.3389/fpls.2016.00426 PMID: 27066058
64. Lee JM, Kim S, Lee JY, Yoo EY, Cho MC, Cho MR, et al. A differentially expressed proteomic analysis in placental tissues in relation to pungency during the pepper fruit development. Proteomics. 2006; 6 (19):5248–59. https://doi.org/10.1002/pmic.200600326 PMID: 16947123
65. Naves ER, de Ávila Silva L, Sulpice R, Araújo WL, Nunes-Nesi A, Peres LE, et al. Capsaicinoids: Pungency beyond *Capsicum*. Trends in plant science. 2019.
66. Bernal MA, Calderon AA, Pedreno MA, Munoz R, Ros Barceló A, Merino de Caceres F. Capsaicin oxidation by peroxidase from *Capsicum annuum* (variety Annuum) fruits. Journal of Agricultural and Food Chemistry. 1993; 41(7):1041–4.
67. Díaz J, Pomar F, Bernal A, Merino F. Peroxidases and the metabolism of capsaicin in *Capsicum annuum* L. Phytochemistry Reviews. 2004; 3(1–2):141–57.

68. Johnson CD, Decoteau DR. Nitrogen and potassium fertility affects jalapeño pepper plant growth, pod yield, and pungency. HortScience. 1996; 31(7):1119–23.

69. Medina-Lara F, Echevarría-Machado I, Pacheco-Arjona R, Ruiz-Lau N, Guzmán-Antonio A, Martinez-Estevez M. Influence of nitrogen and potassium fertilization on fruiting and capsaicin content in habanero pepper (*Capsicum chinense* Jacq.). HortScience. 2008; 43(5):1549–54.

70. Woldemariam SH, Lal S, Zelelew DZ, Solomon MT. Effect of Potassium Levels on Productivity and Fruit Quality of Tomato (*Lycopersicon esculentum* L.). 2018. 2018; 6(1):14. Epub 2017-12-10.

71. Garcia O, Bouige P, Forestier C, Dassa E. Inventory and comparative analysis of rice and Arabidopsis ATP-binding cassette (ABC) systems. Journal of molecular biology. 2004; 343(1):249–65. https://doi.org/10.1016/j.jmb.2004.07.093 PMID: 15381434

72. Hwang J-U, Song W-Y, Hong D, Ko D, Yamaoka Y, Jang S, et al. Plant ABC transporters enable many unique aspects of a terrestrial plant's lifestyle. Molecular Plant. 2016; 9(3):338–55. https://doi.org/10.1016/j.molp.2016.02.003 PMID: 26902186

73. Sidler M, Hassa P, Hasan S, Ringli C, Dudler R. Involvement of an ABC transporter in a developmental pathway regulating hypocotyl cell elongation in the light. The Plant Cell. 1998; 10(10):1623–36. PMID: 9761790

74. Noh B, Murphy AS, Spalding EP. Multidrug resistance–like genes of Arabidopsis required for auxin transport and auxin-mediated development. The Plant Cell. 2001; 13(11):2441–54. https://doi.org/10.1105/tpc.010350 PMID: 11701880

75. Andolfo G, Ruocco M, Di Donato A, Frusciante L, Lorito M, Scala F, et al. Genetic variability and evolutionary diversification of membrane ABC transporters in plants. BMC plant biology. 2015; 15(1):51.

76. 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. Journal of Biological Chemistry. 2004; 279(40):42157–68. https://doi.org/10.1074/jbc.M404502200 PMID: 15277527

77. Kato T, Tabata S, Sato S. Analyses of expression and phenotypes of knockout lines for Arabidopsis ABCF subfamily members. Plant biotechnology. 2009; 26(4):409–14.

78. Borghi L, Kang J, Ko D, Lee Y, Martinoia E. The role of ABCG-type ABC transporters in phytohormone transport. Biochemical Society Transactions. 2015; 43(5):924–30. https://doi.org/10.1042/BST20150106 PMID: 26517905

79. Banasiak J, Jasiński M. Defence, symbiosis and ABCG transporters. Plant ABC Transporters: Springer; 2014. p. 163–84.

80. 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. The Plant Cell. 2011; 23(5):1958–70. https://doi.org/10.1105/tpc.111.083121 PMID: 21628525

81. 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. Proceedings of the National Academy of Sciences. 2011; 108(30):12354–9.

82. Mentewab A, Stewart CN Jr. Overexpression of an Arabidopsis thaliana ABC transporter confers kanamycin resistance to transgenic plants. Nature biotechnology. 2005; 23(9):1177. https://doi.org/10.1038/nbt1134 PMID: 16116418

83. Kuromori T, Ito T, Sugimoto E, Shinozaki K. Arabidopsis mutant of AtABCG26, an ABC transporter gene, is defective in pollen maturation. Journal of plant physiology. 2011; 168(16):2001–5. https://doi.org/10.1016/j.jplph.2011.05.014 PMID: 21696844

84. Xu XM, Møller SG. AtNAP7 is a plastidic SufC-like ATP-binding cassette/ATPase essential for Arabidopsis embryogenesis. Proceedings of the National Academy of Sciences. 2004; 101(24):9143–8.

85. Zhu Y-Q, Xu K-X, Luo B, Wang J-W, Chen X-Y. An ATP-binding cassette transporter GhWBC1 from elongating cotton fibers. Plant physiology. 2003; 133(2):580–8. https://doi.org/10.1104/pp.103.027052 PMID: 12972649

86. Shibata Y, Ojika M, Sugiyama A, Yazaki K, Jones DA, Kawakita K, et al. The full-size ABCG transporters Nb-ABCG1 and Nb-ABCG2 function in pre-and postinvasion defense against Phytophthora infestans in Nicotiana benthamiana. The Plant Cell. 2016; 28(5):1163–81. https://doi.org/10.1105/tpc.15.00721 PMID: 27102667

87. 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. Journal of Biological Chemistry. 2009; 284(48):33614–22. https://doi.org/10.1074/jbc.M109.030247 PMID: 19797057

88. Badone FC, Cassani E, Landoni M, Doria E, Panzeri D, Lago C, et al. The low phytic acid1-241 (lpa1-241) maize mutation alters the accumulation of anthocyanin pigment in the kernel. Planta. 2010; 231 (5):1189–99. https://doi.org/10.1007/s00425-010-1123-z PMID: 20191364

89. 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(2):196–208. https://doi.org/10.3390/plants4020196 PMID: 27135323

90. Goodman CD, Casati P, Walbot V. A multidrug resistance–associated protein involved in anthocyanin transport in Zea mays. The Plant Cell. 2004; 16(7):1812–26. https://doi.org/10.1105/tpc.022574 PMID: 15208386

91. 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. The Plant Cell. 2013; 25 (5):1840–54. https://doi.org/10.1105/tpc.112.102152 PMID: 23723325