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Impact of Some Toxic Metals on Important ABC Transporters in Soybean (*Glycine max* L.)

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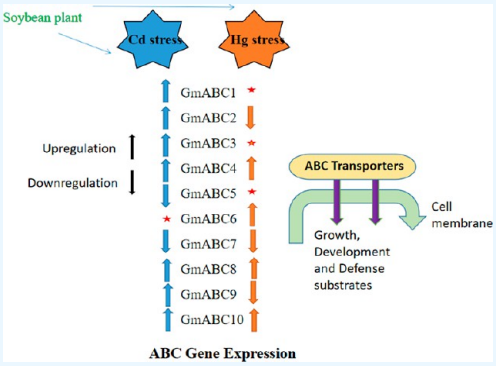
ABSTRACT: In plants, ATP-binding cassette (ABC) transporters facilitate the movement of substrates across membranes using ATP for growth, development, and defense. Soils contaminated with toxic metals such as cadmium (Cd) and mercury (Hg) might adversely affect the metabolism of plants and humans. In this study, a phylogenetic relationship among soybeans' (*Glycine max*) ATP binding cassette (GmABCs) and other plant ABCs was analyzed using sequence information, gene structure, chromosomal distribution, and conserved motif-domain. The ontology of GmABCs indicated their active involvement in trans-membrane transport and ATPase activity. Thirty-day-old soybean plants were exposed to 100 μM $CdCl_2$ and 100 μM $HgCl_2$ for 10 days. Physiological and biochemical traits were altered under stress conditions. Compared to Control, GmABC transporter genes were differentially expressed in response to Cd and Hg. The qRT-PCR data showed upregulation of seven ABC transporter genes in response to Cd stress and three were downregulated. On the other hand, Hg stress upregulated four GmABC genes and downregulated six. It could be concluded that most of the ABCB and ABCG subfamily members were actively involved in heavy metal responses. Real-time expression studies suggest the function of specific ABC transporters in Cd and Hg stress response and are helpful in future research to develop stress-tolerant varieties of soybean.



INTRODUCTION

ABC (ATP-binding cassette) transporters are dynamic proteins that play a pivotal role in the translocation of solutes across cell membranes. Found in both prokaryotes and eukaryotes, they are generally of three types and are characterized as type 1, type 2, and type 3. Type 1 and type 2 ABC importers transport a large variety of nutrients, biosynthetic precursors, trace metals, and vitamins.[1] ABC transporters of plants are present in eight subfamilies using the nomenclature: A, B, C, D, E, F, G, and I.[2,3] It has been suggested that ABC transporter proteins in plants are associated with their terrestrial and sessile lifestyle, which makes them more diversified and helps them to cope with abiotic stress.[4] Structurally, ABC transporters comprise four domains: two cytoplasmic nucleotide-binding domains (NBDs) and two highly hydrophobic transmembrane domains (TMDs) with highly conserved motifs. The NBDs use ATP for the import or export of various substrates across membranes.[5]

In plants, ABC transporters emerged to be a potential way to clear out toxic ions. To resist heavy metal toxicity, the defense mechanism via ABC transporters involved heavy metal uptake, binding to phytochelatins/metallothioneins, activation of specific ABC transporters, sequestration of compound conjugates in the large central vacuole, and release into the apoplast in a process known as internal or external excretion.[6,7] These transporters were actively involved in the process of transporting toxic metal ions and protecting plants from their harmful effects.[8,9]

In *Arabidopsis*, under metal stress, two ABC transporters *AtABCC1* and *AtABCC2* were identified as playing roles in the vacuolar Phytochelatin (PC) transport.[10] Besides, in response to pathogens and toxic ions, the plasma membrane-intrinsic full-size transporter protein *AtABCG36/AtPDR8* was found to be involved.[11] Overexpression of a mitochondrial ABC transporter *AtATM3* conferred resistance to Pb and Cd stress by enhancing translocation to the shoots.[12]

Heavy metal pollution in the environment is a critical health issue for plants and animals.[13] Cadmium (Cd) and mercury (Hg) are two different environmental pollutants toxic to plants.[14−16] Several reports on ABC transporters revealed their important roles in imparting tolerance to both Cd and Hg.[17] These transporters are mainly known to contribute to various physiological and metabolic processes of plants which make

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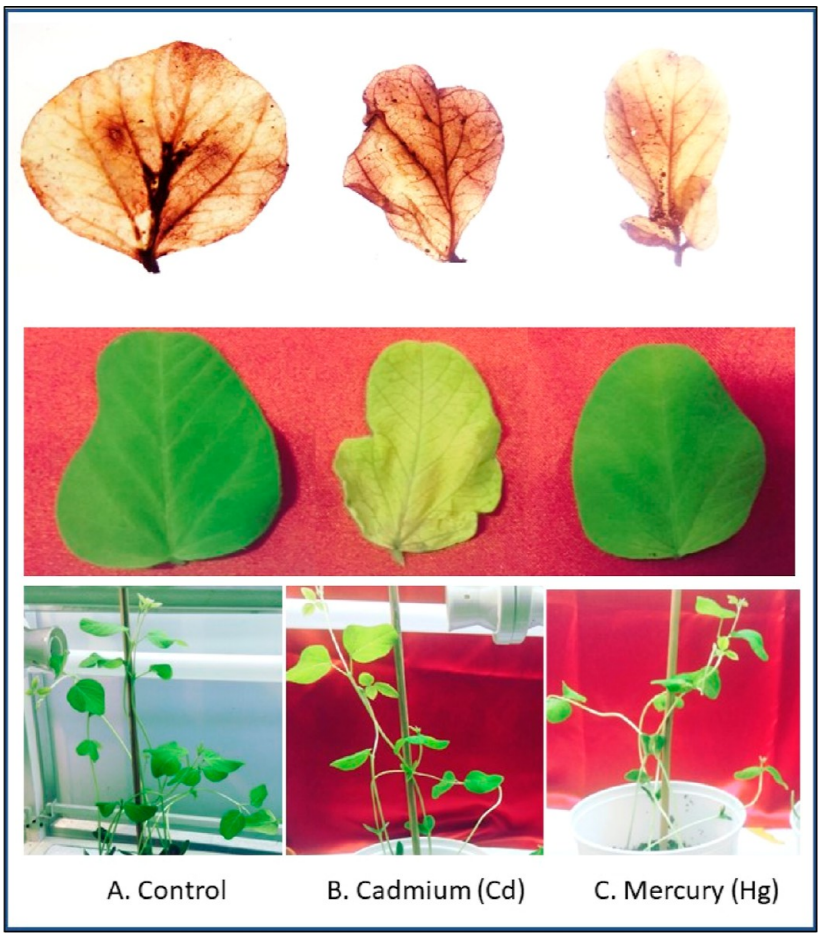

Figure 1. Forty-days-old plants of soybean: (A) control plant, (B) Cd-treated plant (100 μM $CdCl_2$), and (C) Hg-treated plant (100 μM $HgCl_2$). It is noteworthy that first row showed histochemical detection of H_2O_2 in leaves, Cd accumulated more H_2O_2, second row denoted Cd and Hg treated leaves and third row indicated effect of Cd and Hg on fully grown plants in pots.

them adapt various challenging environmental conditions and deal with biotic and abiotic stress.[18] Furthermore, they are involved in the transportation of secondary metabolites which were related to plant defense against abiotic stress. Some investigations on Cd toxicity proved that ABCC-type transporters were significantly involved in the translocation of Cd into the central vacuole for detoxification purposes.[19]

Soybean (*Glycine max* L.) is the world's most important staple and oilseed legume; two-thirds of the world's protein requirement is met by soybean for livestock feeding.[20] Soybean is thus a major crop of economic importance being a source of protein for humans and animals. Therefore, it is an important model crop for research.

As a mere fact, soybean cultivating lands suffer from numerous challenges due to abiotic stresses such as drought, salinity, metal stress, and water submergence costing it the level of quality and productivity.[21] There are reports of an increase in levels of toxic metals in the soil of soybean cultivating regions.[15,22] Such toxic metal ions enter the plant cells by a similar uptake process as nutrient ions, competing for absorption and ultimately adversely affecting the metabolism to limit crop production.[23] Cadmium (Cd) and mercury (Hg) were found to be highly hazardous toxic metals.[24,25]

Even at very low concentrations, Cd can severely change plant enzyme activities and ultimately cause stress.[26,27] Another very potent toxic metal is mercury (Hg) which causes serious crop destruction in plants, alteration in RNA expression, DNA methylation, modifications in histones, and visible injuries and physiological disorders in humans.[22,28] It is preferentially bound with ligands of sulfur and enters the cell through ionic channels.[29] The consequence of heavy metals which altered physiological and biochemical traits in plants was also studied in this study.

Exposure to Cd changed the expression pattern of various key genes involved in the regulatory mechanism of plants.[15,30,31] Besides interacting directly to bring changes in metabolism and physiology, metals' stress influences the expression of certain genes, often to induce proper acclimation mechanisms and cellular reprogramming to minimize damage to the plants.[7,32]

To date, the response of ABC transporter genes to heavy metal stress has not been assessed and analyzed in soybean. To reveal the possible role of ABC transporter genes in combating heavy metal stress in soybean we systematically identified some selective novel representative ABC transporter genes (GmABCs) through bioinformatics tools that were related to defense mechanisms based on their unique motif-domain organization and comparatively analyzed their expression patterns in response to Cd and Hg stress through (qRT)-PCR. The characterization of soybean ABC transporter genes (GmABCs) was done by analyzing the biophysical properties of their proteins. Other genetic relations were analyzed using chromosomal distribution, gene structure, expression analysis, and phylogenetic analysis. Such a study is expected to help

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understand the evolutionary relationships of soybean ABC transporter genes with other plants' ABCs and their role in heavy metal stress management. This study surely provides an important link between some ABC transporters of soybean with heavy metal tolerance.

MATERIALS AND METHODS

Plant Growth and Stress Treatment. Soybean seeds of a high-yielding popular Indian variety JS-335 (obtained through proper permission from Directorate of Soybean Research, JNKVV, Jabalpur, India) were washed and sterilized with 1% sodium hypochlorite solution followed by 20 rinse-wash by sterilized double distilled water (DDW). Seeds were germinated on Whatman filter paper in the dark for 3 days and transferred to soilrite filled in 7 in. pots which were divided into three sets as Control (without stress), cadmium (Cd), and mercury (Hg) treatment for further growth. These pots were kept inside a growth chamber at 28 °C and 16 h light and 8 h dark cycles. 30 days-old plants were induced for stress, viz., pots in triplicate were supplied with 100 μM $CdCl_2$ and another set of pots were supplied with 100 μM $HgCl_2$ dissolved in Hoagland nutrient solution for 10 days. After 40 days of germination, the plants were carefully picked up and washed with distilled water (Figure 1). Leaves were harvested from all sets of plants, frozen in liquid nitrogen, and stored at −20 °C for further study.

Identification and Classification of ABC Transporter Genes in Soybean. In this study, genes of 10 selective ABC transporters (7 on the plasma membrane, 1 cytoplasm, and 2 vacuolar membranes) were shortlisted from the soybean genome using the SoyKB database (soykb.org) and Phytozome server (https://phytozome.jgi.doe.gov/pz/portal.html) having different types of recognized transporter domain.[33] These were ABC transporter domain (PF00005), the ABC-2 transporter domain (PF01061), and ABC transporter transmembrane region domain (PF00664) and serially named SoyKB gene IDs as, *Glyma.01G008200.1* (herein GmABC1), *Glyma.02G038800.1* (herein GmABC2), *Glyma.05G145000.2* (herein GmABC3), *Glyma.01G016500.1* (herein GmABC4), *Glyma.16G172400.5* (herein GmABC5), *Glyma.15G011800.1* (herein GmABC6), Glyma.13G002500.1 (herein GmABC7), *Glyma.08G070700.1* (herein GmABC8), *Glyma.05G019400.1* (herein GmABC9), and *Glyma.08G070800.1* (herein GmABC10). In addition, the corresponding genome and coding sequences along with their chromosomal positions were also retrieved from Phytozome[34] and the SoyKB database.[3] Physicochemical characteristics of ABC proteins and amino acid sequences were predicted by the ProtParam tool (web.expasy.org), including molecular weight (MW), instability index, alignment length, and theoretical isoelectric point (pI).[35]

Subcellular Localization, Conserved Motifs, and Gene Structure Analysis of GmABC Transporters. Subcellular localization of the selected ABC transporter proteins was obtained from the ProtComp9.0 server (www.softberry.com).[36] The domain information of all selected gene sequences was retrieved from SoyKB and Phytozome portals. Conserved motif analysis of these GmABCs was performed by the Multiple Em for Motif Elicitation (MEME Suite4.11.1)[37] and motif locations were retrieved from the Phytozome server. Configurations of intron/exon of GmABC genes were determined through Gene Structure Display Server 2.0 (GSDS 2.0) and obtained both genome and coding sequences.[38]

Chromosomal Location and Homology Modeling of GmABC Transporter Genes. Information on chromosomal location of selective GmABC transporter genes was retrieved from *G. max* Wm82.a2.v1 genome in the Phytozome database through BLASTN searches. Chromosomal locations of the ABC transporter genes were performed and mapped based on information gathered from the Phytozome database.[9] In addition, corresponding coding sequences and protein domains were also retrieved and visualized using Phytozome. The three-dimensional (3D) structures of GmABC transporter genes were generated by intensive protein homology modeling using the Phyre2 server (Protein Homology/Analogy Recognition Engine; http://www.sbg.bio.ic.ac.uk/phyre2).

Gene Ontology and Statistical Analysis. Gene ontology of GmABCs was carried out using the agriGO database (http://bioinfo.cau.edu.cn/agriGO) and statistical analysis was carried out using MetaboAnalyst 3.0.[39] All data were log2 transformed for hierarchical clustering, principal component analysis (PCA), and partial least squares discriminant analysis (PLS-DA).[15] All data were presented as the mean ± standard error (SE). The measurements were taken from three biological replicates

Primer Designing. Primers were designed and synthesized for housekeeping gene, Plant actin with gene ID KM065998.1 (Internal control) and 10 selected soybean ABC transporter genes, i.e., *Glyma.01G008200.1*, *Glyma.02G038800.1*, *Glyma.05G145000.2*, *Glyma.01G016500*, *Glyma.16G172400.5*, *Glyma.15G011800.1*, *Glyma.13G002500.1*, *Glyma.08G070700.1*, *Glyma.05G019400.1*, and *Glyma.08G070800.1*, using Primer3 plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Supporting Information, Table S1).

RNA Isolation and Quantification. Total RNA from leaves of Control, Cd-, and Hg-treated plants was isolated using an RNA isolation kit (Chromous, India). RNA was quantified using BioPhotometer Plus (Eppendorf, Germany).

First-Strand cDNA Synthesis and Quantitative RT-PCR. 1.0 μg of total RNA was reverse transcribed using an M-MuLV RT-PCR kit (Merck, Germany). The first strand was used for qRT-PCR.

Quantitative Real-Time PCR (qRT-PCR). The qRT-PCR was done on three biological replicates using SYBR green master mix, cDNA, Actin (internal control), and ABC transporters gene-specific primers with a total volume of 20 μL on a Rotor Gene 6000 real-time rotary analyzer (Corbett Research, Australia). For data normalization, CT values of genes were subtracted from the CT value of the reference gene (ΔCT) and relative quantification (2-ΔΔCT) was calculated by subtracting ΔCT of interest from ΔCT of the reference gene.

Phylogenetic Analysis of ABC Transporter Genes of Soybean with Other Plants. To determine the phylogenetic relationship of soybean ABC transporter genes with other plants a total of 100 (cutoff point) ABC transporter protein sequences were retrieved falling in nine plant species including *Glycine max*, *Arabidopsis thaliana*, *Mucuna pruriens*, *Oryza sativa japonica*, *Capsicum annuum*, *Theobroma cacao*, *Zea mays*, *Vitis vinifera*, and *Cucumis sativus*. These were the only plants for which the ABC transporter protein sequences were available in NCBI at the time of study and they all are important plants with established economic value and hence

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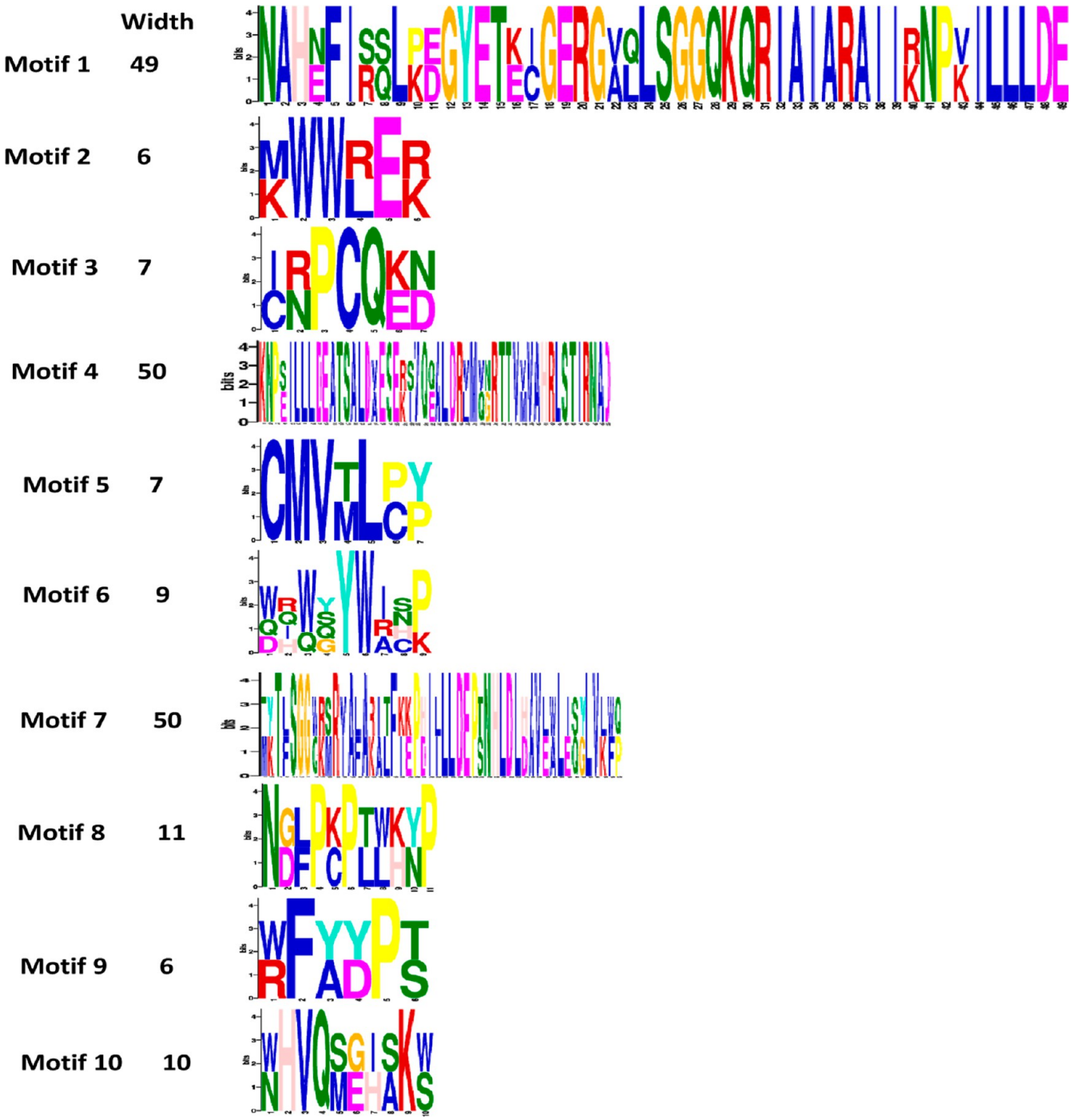

Figure 2. Conserved amino acids in 10 motifs of ABC transporter proteins in soybean with their width and length obtained through MEME 5.4.1 server (https://meme-suite.org).

serve as model plants. Gene names, Gene IDs, LOCI, Accession Numbers, subfamilies, and functions of these ABC transporters are listed in Supporting Information, Table S2A–J. Multiple sequence alignments of ABC genes amino acid sequences were performed with ClustalW[40] using default parameters. An unrooted phylogenetic tree was constructed based on ABC transporter proteins using the software MEGA5.04 via the neighbor-joining method.[41] In the phylogenetic tree, the degree of support for a particular grouping pattern was evaluated using the bootstrap (1000 replicates) value.

RESULTS

Identification of ABC Transporter Genes in Plants. Of the analyzed 100 ABC transporters (Supporting Information, Table S2A–J), 40 were found to be of subfamily ABCG, 29 of ABCB, 21 of ABCC, 4 of ABCA, 4 of ABCI, and 2 of ABCF subfamily. Generally, ABC transporters were found to be involved in the function of the abiotic and biotic stress response, defense mechanisms, eye pigment transport, and drug resistance. The listed functions of these transporters indicate that most of the genes of ABCB and ABCG

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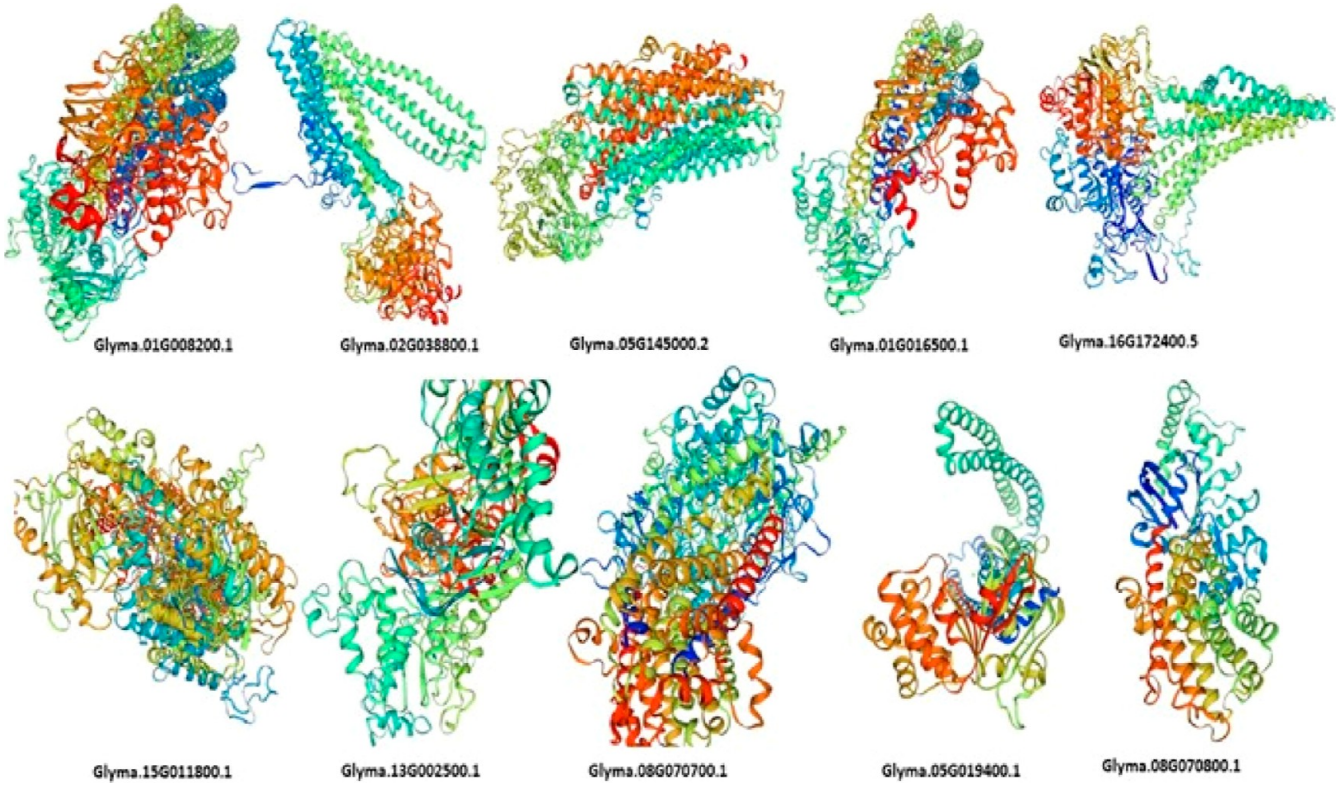

Figure 3. Predicted three-dimensional structure of ABC transporter proteins of soybean obtained through Phyre2 server (http://www.sbg.bio.ic.ac.uk > phyre2) showing great diversity in their structural forms.

subfamilies were found to be involved in stress tolerance in plants.

Identification of Some ABC Transporter Genes in Soybean. A total of 10 important soybean ABC transporter genes which give maximum hits were identified from SoyKB database such as *Glyma.01G008200.1* (herein GmABC1), *Glyma.02G038800.1* (herein GmABC2), *Glyma.05G145000.2* (herein GmABC3), *Glyma.01G016500.1* (herein GmABC4), *Glyma.16G172400.5* (herein GmABC5), *Glyma.15G011800.1* (herein GmABC6), *Glyma.13G002500.1* (herein GmABC7), *Glyma.08G070700.1* (herein GmABC8), *Glyma.05G019400.1* (herein GmABC9), and *Glyma.08G070800.1* (herein GmABC10). They consist of different types of functional domains such as the ABC transporter domain (PF00005), the ABC-2 transporter domain (PF01061) and the ABC transporter trans-membrane region domain (PF00664).[33] Selected soybean ABC transporter genes belong to subfamilies ABCB, ABCC, ABCF, and ABCG. GmABC1, GmABC2, GmABC4, and GmABC9 are from ABCB subfamily; GmABC3 and GmABC5 from ABCC subfamily; GmABC6, GmABC8, and GmABC10 from ABCG subfamily; and GmABC7 are from ABCF subfamily.

Gene Information and Chromosome Mapping. The mRNA's start point and end-point information of selected genes are provided in Supporting Information, Table S3. All GmABC transporter genes had positive strands except GmABC7 which had negative strands. The cDNA length varied from 2482 bp (GmABC8) to 4817 bp (GmABC6) and CDS (coding sequence) length varied from 1872 bp (GmABC10) to 4539 bp (GmABC6). The chromosomal distributions of GmABC were determined in the soybean genome as shown in Supporting Information, Figure S1. Seven out of the 20 chromosomes comprised these ABC transporter genes. Almost all GmABC genes were lying at the terminals of chromosomes. Chromosomes 1, 5, and 8 possessed double GmABC genes while each of the remaining four chromosomes (chromosome 2, 13, 15, and 16) contained only a single GmABC. On chromosome 8, a tandem gene duplication event occurred between GmABC8 and GmABC10. ABC transporter genes that were found on the same chromosomes are GmABC1 and GmABC4 on chromosome 1, GmABC8 and GmABC10 on chromosome 8, and GmABC3 and GmABC9 on chromosome 5.

Physicochemical Analysis of GmABC Transporter Proteins. The physicochemical investigation of GmABC transporter proteins described that the length of amino acid sequences, isoelectric point (pI), and molecular weight (MW) values varied among them. The lengths of peptides length ranged from 624 amino acids to 1513 amino acids, MW ranged from 68581.96 to 170991.72 kg/mol, and pI ranged from 5.70 to 8.76. The alignment length varied from 445 amino acids to 1397 amino acids. The instability index revealed that all GmABC transporter proteins were stable except GmABC2, GmABC5, and GmABC8. Subcellular localization of GmABC proteins determined that out of all, two GmABC transporter genes GmABC2 and GmABC9 were localized in the vacuole, while GmABC7 was localized in the cytoplasm. The remaining GmABC transporter genes were localized in the plasma membrane (Supporting Information, Table S4).

Conserved Motifs and Domain Analysis of GmABC Transporter Proteins. Several motifs were widely distributed in the GmABC transporter proteins, 10 conserved motifs were identified in these proteins (motifs 1−10) (Figure 2). The width of motifs varied from 6 to 50 bp. Most of the motifs

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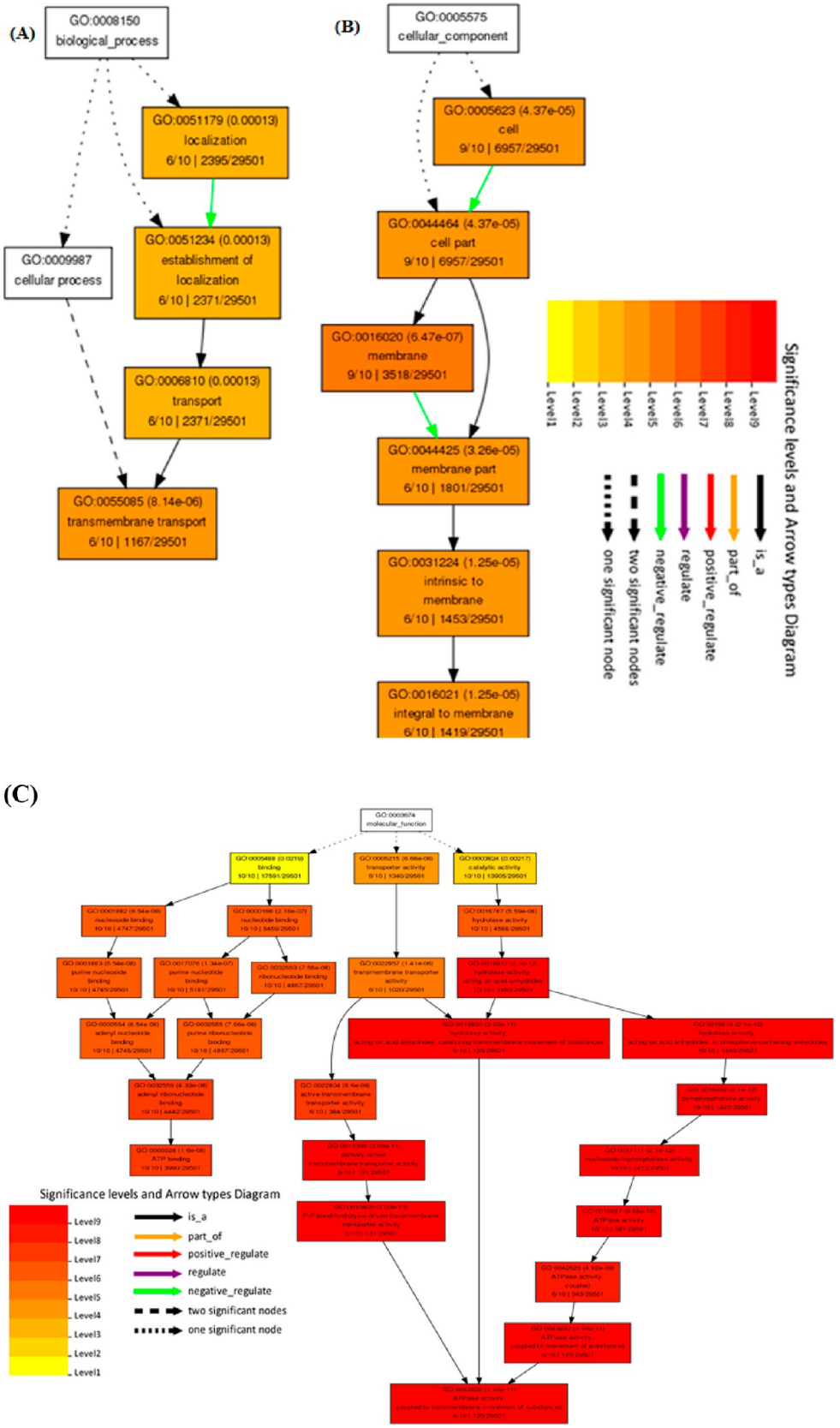

Figure 4. (A–C) GO analysis of GmABC transporter genes showing (A) biological functions, (B) cellular components, and (C) molecular functions. Photographs were developed through Gene Ontology Resource (http://geneontology.org).

were of the same width. The *p*-value of motifs varied from 1.15 × 10^{-54} to 7.16 × 10^{-9}. All GmABCs had domains of TMD-NBD and NBD-TMD organization. Only GmABC6 was found to have accessory/regulatory domains. Results indicated that

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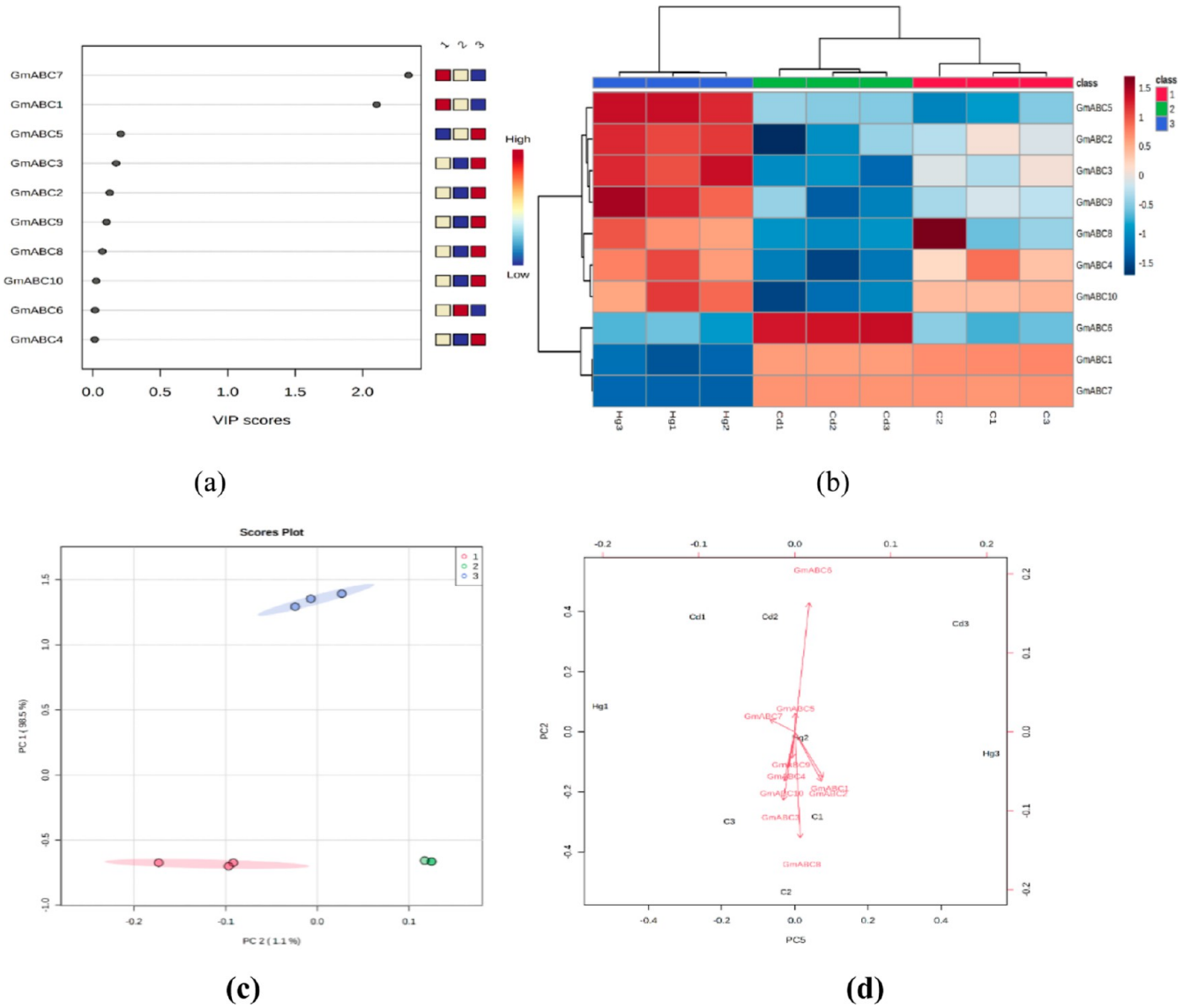

Figure 5. Multivariate Data analysis of differentially expressed GmABC transporter genes developed by MetaboAnalyst 3.0 (https://www.metaboanalyst.ca) software showing (a) heatmap of differentially expressed GmABC transporter genes in the case of Control or heavy metal treated (C1, C2, and C3 as Control, Hg1, Hg2, and Hg3 as mercury treated, and Cd1, Cd2, and Cd3 as cadmium treated condition), (b) loadings plot, (c) VIP plot, and (d) biplot.

these extremely conserved motifs were probably required for specific translocation of substrates through ATP hydrolysis.

KOG Id and Pfam Id of selected GmABC and their functions are specified in Supporting Information, Table S5. Pfam described that all domains have an ABC transporter transmembrane region. The Pfam domain of all transporter genes of soybean was related to peptide exporter, pleiotropic drug resistance proteins, multidrug/pheromone exporter, and multidrug resistance-associated protein and xenobiotics transporting ATPase, defense mechanisms, and biotic and abiotic stress tolerance (Supporting Information, Figure S2).

Intron−Exon Configurations and Gene Structure. Intron−exon configurations of GmABC transporter genes were by aligning the cDNA sequences with the corresponding genome DNA sequences. The gene structure displayed that intron numbers among these GmABC genes ranged from 5 to 23. Two genes GmABC2 and GmABC6 exhibited the highest intron number, i.e., 17 and 23, respectively, whereas GmABC3 had only five introns (Supporting Information, Figure S3). In addition, two GmABC genes (GmABC1 and GmABC8) exhibited similar number of introns. The remaining GmABC genes exhibited diverse intron/exon organization patterns.

Homology Modeling of GmABC Transporter Proteins. Phyre2 server was used for homology modeling and prediction of three-dimensional structures of selected GmABC transporter proteins. Remarkably, these 10 ABC proteins mostly belonged to different subfamilies. These protein structures were modeled at >90% confidence (Figure 3). Predicted structures were extremely reliable and helpful for understanding molecular function.

Gene Ontology of GmABC Transporters. Analysis of gene ontology displayed functional configuration of GmABC transporter genes (Figure 4A−C). Biological functions demonstrate the directed transport of substances from one place to another in the cell and an overall increase in localization of substrate and transport activity (Figure 4A). Functions of cellular components showed an increase in membrane and cellular activity (Figure 4B), whereas molecular

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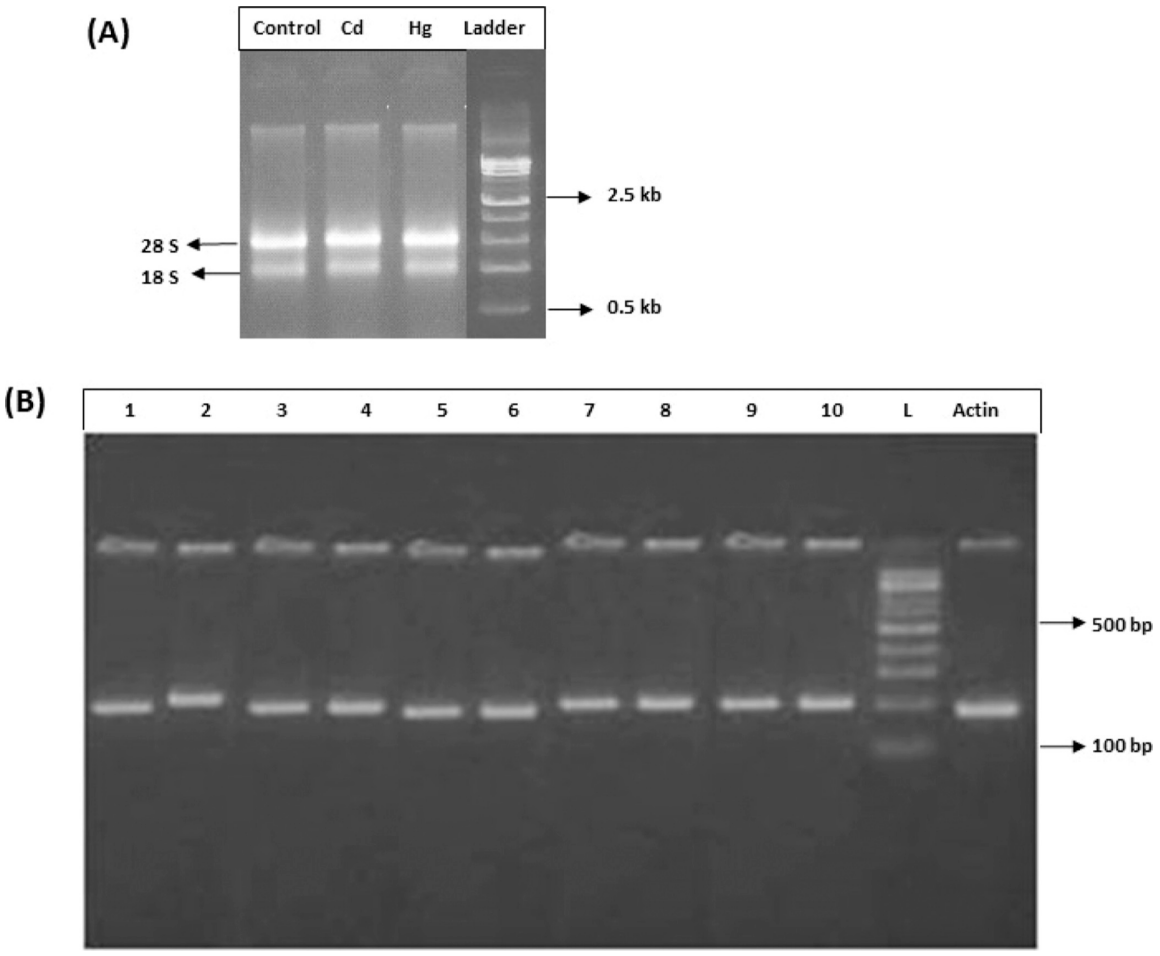

Figure 6. (A) 1% Agarose gel showing isolated RNA bands of Control and stress (Cd and Hg)-treated plant with 500 bp DNA ladder, (B) PCR amplified products of GmABC transporter genes in 2% agarose gel (lanes 1−10). Membrane edges of gels were not visible due to cropping of gels as both the RNA isolation and RT-PCR bands run on the same gel simultaneously due to the lack of time constraints. Lane description; (1) PCR amplicon of control V/s GmABC5, (2) PCR amplicon of control V/s GmABC4, (3) PCR amplicon of control V/s GmABC8, (4) PCR amplicon of control V/s GmABC7, (5) PCR amplicon of control V/s GmABC9, (6) PCR amplicon of control V/s GmABC3, (7) PCR amplicon of control V/s GmABC10, (8) PCR amplicon of control V/s GmABC6, (9) PCR amplicon of control V/s GmABC2, and (10) PCR amplicon of control V/s GmABC1, L- 100 bp DNA ladder, and actin gene as an internal control.

functional components displayed an increase in transmembrane transporter activity, nucleotide binding activity, ATPase activity, and hydrolase activity (Figure 4C). These results indicated the active participation of ABC transporter proteins in diverse biological processes as they are mainly involved in transportation, the establishment of localization, nucleotide binding, hydrolase activity, ATPase activity, assembly of cellular constituents, and metabolic process. Cellular localization prediction showed that these GmABC proteins are equally localized between the cell part and cellular membrane (Supporting Information, Figure S4).

Differential Expression of GmABC Transporter Genes and Multivariate Data Analysis. The interdependence of GmABC genes under Cd and Hg stress was analyzed by multivariate data analysis of qRT-PCR data (Figure 5a−d). Hierarchical clustering of genes was performed and a heat map was generated to analyze their differential expression and correlation changes between gene profiles (Figure 5a). Hierarchical clustering displayed variations between differentially expressed GmABC genes in control and heavy metal (Cd and Hg)-treated plants. The principal component analysis (PCA) scores plot (Figure 5b) informed about data and compared the differentially expressed ABC genes in the soybean plant. Identification of maximum changes in abundance of genes was described by the VIP plot which was constructed from the PLS-DA loadings plot (Figure 5c).

In the VIP plot, a total of 10 genes with VIP scores (0.10−3.0) included 10 GmABC transporters. Biplot provided information about a correlation among these genes. GmABC genes (1, 2, 3, 4, 8, 9, and 10) clustered together and showed a positive correlation among them and responded positively toward Cd stress (Figure 5d), whereas GmABC (5, 6, and 7) clustered together in the opposite direction and showed negative correlations with other genes. The long vector of GmABC6 toward the opposite direction revealed a positive response toward Hg stress. These plots suggested that GmABC transporter genes (1, 2, 3, 4, 6, 8, 9, and 10) might play an important role in heavy metal stress management.

qRT-PCR-Based Comparative Gene Expression Analysis of GmABC Transporter Genes. Total isolated RNA was found to be 283 μg/g fresh weights (FW) in control plants. In response to Cd and Hg stress, the concentration of RNA was decreased to 211 μg/g and 226 μg/g, respectively (Figure 6A), which might be due to the oxidation of RNA

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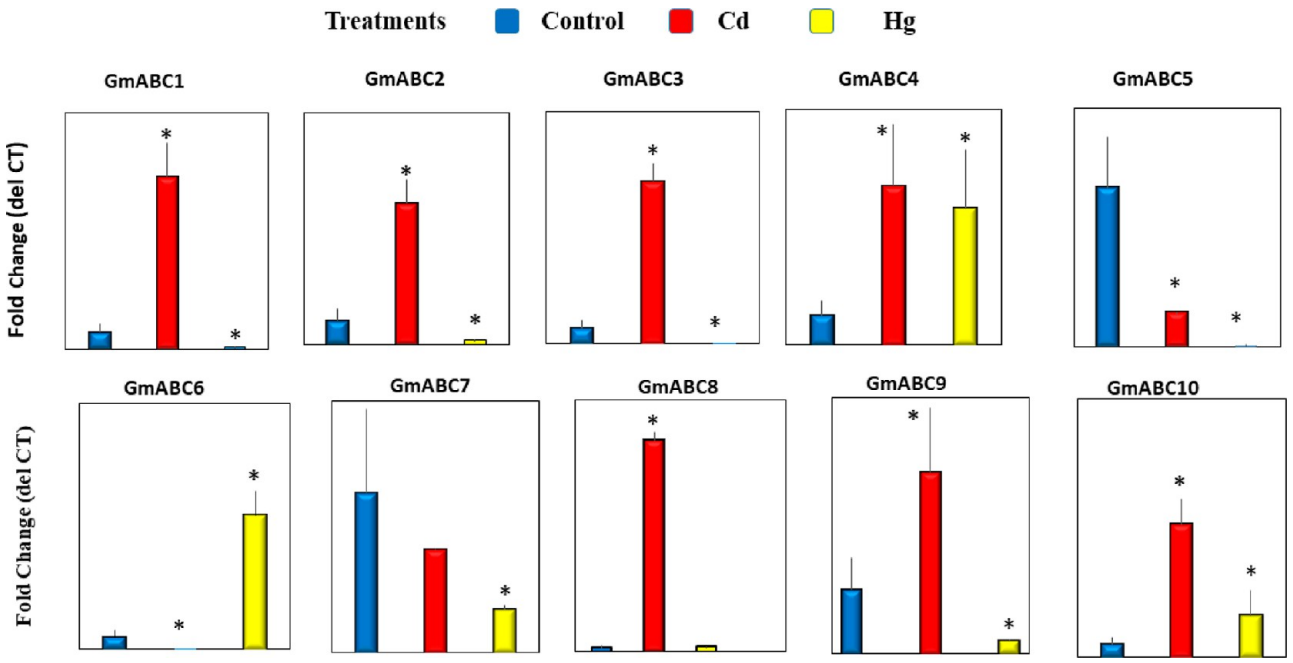

Figure 7. Relative gene expression profiles of soybean ABC transporter genes (GmABC) in control (-Cd,-Hg) and in response to Cadmium (100 μM) and Mercury (100 μM) treatments using qRT-PCR in which actin is used as an internal control. Asterisks indicate $p < 0.05$ (t-test). All data are represented as Mean ± standard error (SE). The measurements were made using three independent biological replicates.

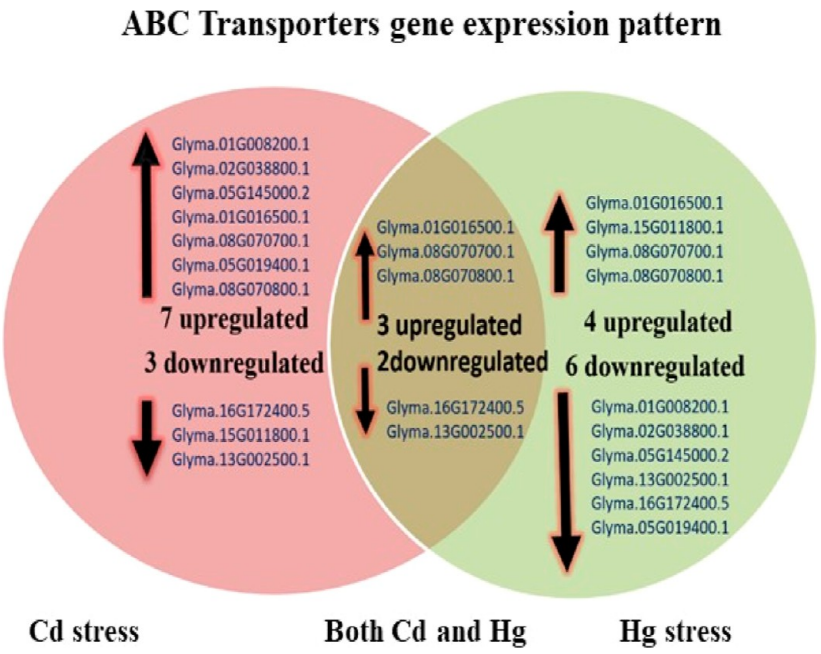

Figure 8. Venn representation of expression pattern of ABC transporter genes of soybean in response to Cd and Hg stress treatment.

molecules when exposed to heavy metals. RT-PCR amplified products were electrophoresed using 2% Agarose gel (Figure 6B and Supporting Information, Figure S5).

The qRT-PCR-based relative expression analysis (Figure 7) revealed that, in response to Cd stress, most of the soybean ABC transporter gene (GmABCs) expression levels were upregulated in comparison to non-treated plants such as GmABC1 (10-fold greater expression), GmABC2 (6-fold), GmABC3 (10-fold), GmABC4 (5.4-fold), GmABC8 (≥50 fold), GmABC9 (2.8-fold), and GmABC10 (10.3-fold), but downregulation was observed in GmABC5, GmABC6, and GmABC7. When exposed to Hg stress, there is a significant upregulation in four soybean ABC transporter genes as compared to control. GmABC4 (4.6-fold), GmABC6 (10.8-fold), GmABC8 (1.3-fold), and GmABC10 (3.2-fold); however, downregulation displayed in GmABC1, GmABC2, GmABC3, GmABC5, GmABC7, and GmABC9.

Results indicated that 7 ABC transporter genes out of 10 were significantly upregulated in response to Cd stress, and only 3 were downregulated, comparatively, Hg stress considerably upregulated 4 GmABC genes and downregulated 6 other GmABCs. However, in response to both Cd and Hg,

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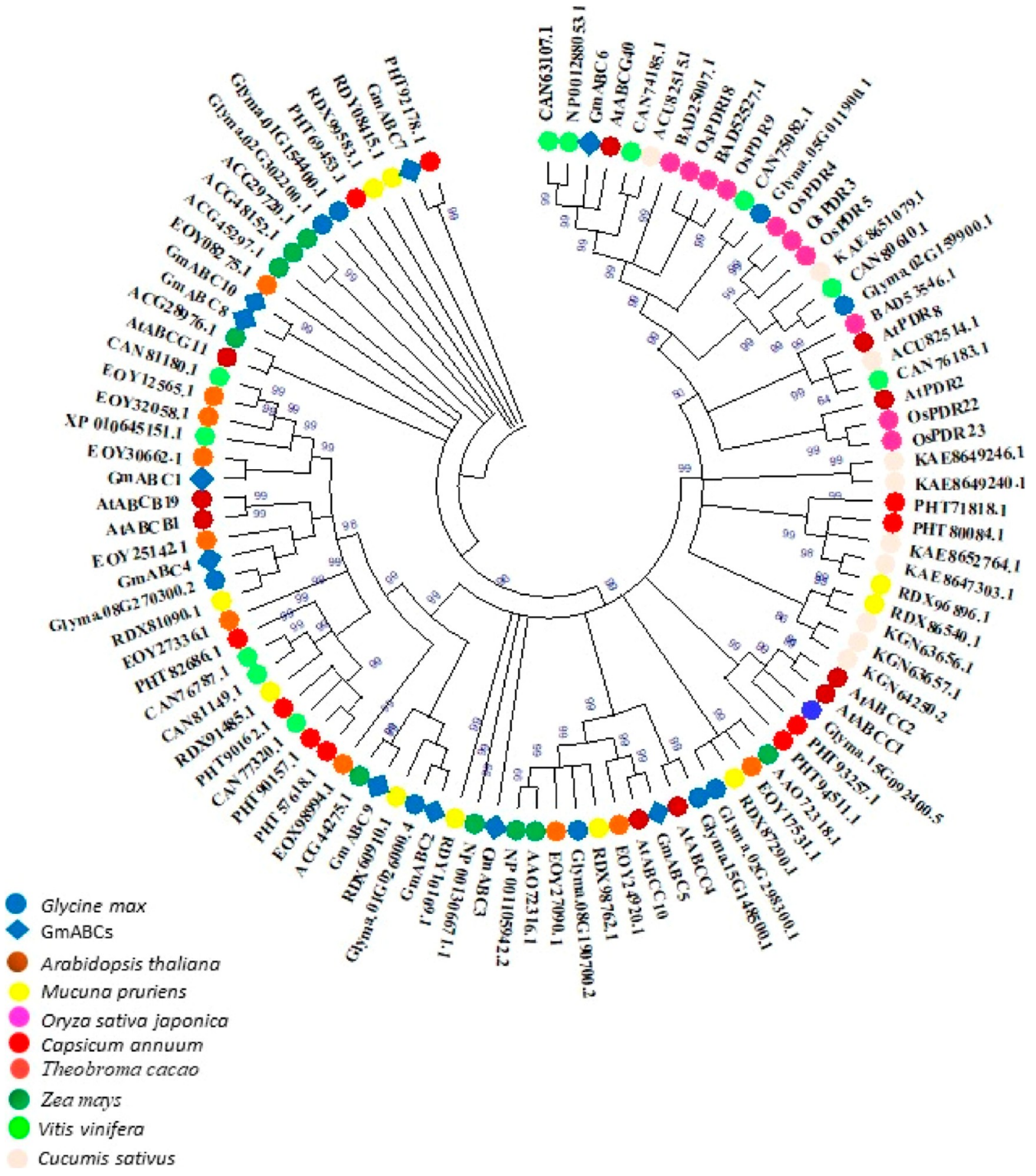

Figure 9. Phylogenetic tree of important ABC transporter proteins developed using MEGA5.04 (https://www.megasoftware.net) from soybean and other plant species. Blue circle dots represent-ABC transporter genes in *Glycine max* including (GmABCs in square dots), brown: ABC transporter genes, yellow: *Mucuna pruriens* ABC transporter genes, pink: *Oryza sativa japonica* ABC transporter genes, red: *Capsicum annuum* ABC transporters, orange: *Theobroma cacao* ABCs, dark green: *Zea mays*, light green: *Vitis vinifera*, and cream: *Cucumis sativus* ABC transporter genes.

there is significant upregulation of GmABC4, GmABC8, and GmABC10 genes and downregulation displayed in only two GmABCs, i.e., GmABC5 and GmABC7 (Figure 8).

Phylogenetic Analysis of ABC Transporter Genes in Plants. To study the phylogenetic relationship among soybean ABC transporters and with other identified plants ABCs from *A. thaliana*, *M. pruriens*, *O. sativa japonica*, *C. annuum*, *T. cacao*, *Z. mays*, *V. vinifera*, and *C. sativus* a phylogenetic tree was constructed from the protein sequences of ABC genes listed in Supporting Information, Table S1A–J. Phylogenetic analysis revealed that GmABCs were spread in all directions in a tree and displayed divergence. GmABC1 grouped with *V. vinifera*,

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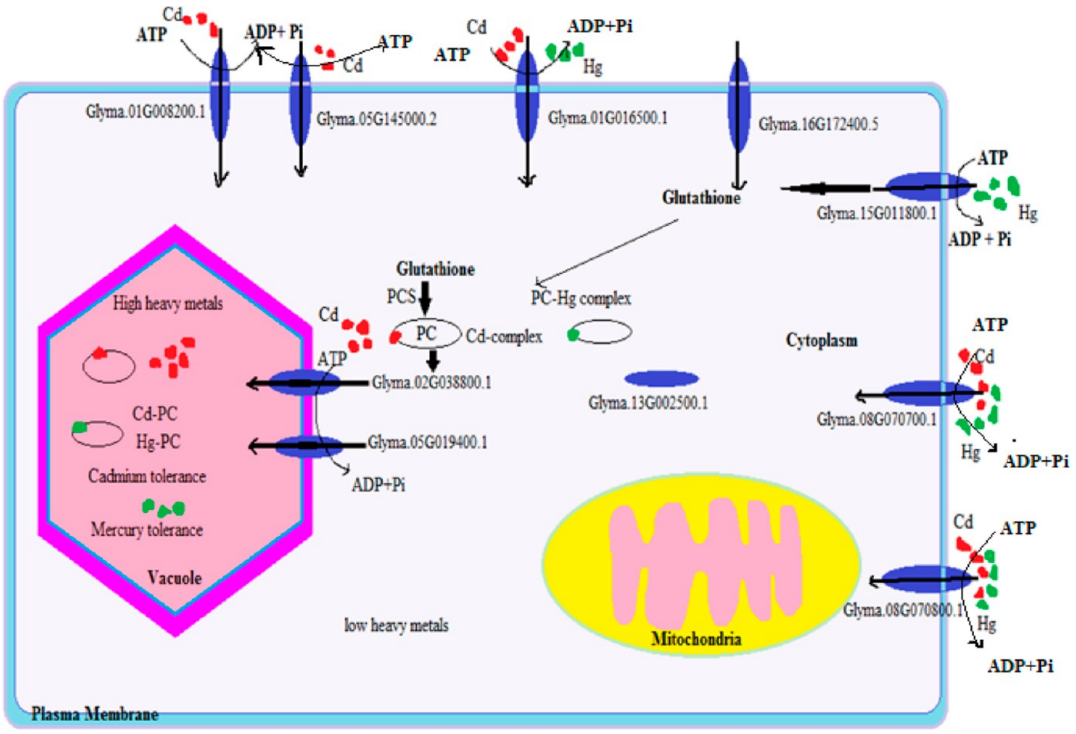

Figure 10. Detoxification of heavy metal Cd and Hg in soybean plant cells which involved ABC transporter genes. PC: phytochelatin, PCS: phytochelatin synthase, red dots: Cd ions, and green dots: Hg ions. Seven ABC transporter genes were located on the plasma membrane with two located on the membrane of the vacuole and a single gene located in the cytoplasm of the plant cell.

CAN81180.1, and cladded with *T. cacao*, EOY30662.1. Tree revealed that GmABC8 and GmABC10 fall on the same clade. Sequence comparison indicated that the GmABC transporters genes were more closely related to one another than ABC transporters from other plants. In addition, six GmABC (1, 2, 4, 5, 6, and 9) were clustered with other plants (Figure 9). However, GmABC2 and GmABC9 fall in the same group, and GmABC9 cladded with *Z. mays*, *ACG44275.1*, and GmABC2 linked with *RDX60910.1* of *M. pruriens*. GmABC6 grouped with *V. vinifera* and clade with *ATABCG40*. Similarly, GmABC4 grouped with *A. thaliana*, *ATABCB19*, and clade with *Mucuna*, *RDX81090.1* (Figure 9).

DISCUSSION

The growth and productivity of the soybean crop are adversely affected by heavy metal stress. Humans might also be exposed to such metals through the consumption of plant products that had accumulated in due process of phytoextraction.[21,42] However, plants have developed various detoxification mechanisms to mitigate the harmful effects of heavy metals. Movement across the plasma membrane through transporter proteins could play an important role. ABC transporters are such protein channels for such action and are involved in metal transport and imparting stress tolerance.

In our study, the function of some ABC transporters in soybean was analyzed through gene expression data as studied in response to Cd[43] and Hg.[44] Although more than 400 ABC transporters have been characterized in soybean so far, 10 of them were shortlisted based on their important roles at different cellular locations. Selected GmABC transporters related to some of the best-characterized subfamilies of ABC which were reported to be highly involved in the transportation of toxic compounds out of the cell such as multidrug resistance proteins (MDR/ABCB) subfamily, multidrug resistance-associated proteins (MRP/ABCC), and pleiotropic drug resistance proteins (PDR/ABCG) subfamily.[3,45−47] ABC transporter gene families have been extensively characterized in other plants, whereas Soybean comprises more ABC transporters because it has undergone a significant number of tandem and segmental duplication of genes which led to a large diverse family of ABC transporters in silico analysis greatly helping in such studies.[48]

GmABCs selected in this study were found widely distributed in plasma and vacuolar membranes. Diverse physicochemical properties among GmABC transporters were observed besides immense variation in size and gene structure which could be attributed to their location in chromosomes making them highly variable.[49]

Chromosome distribution revealed that these genes were unevenly distributed among 20 chromosomes and most of the genes were located on the same chromosomes. GmABC which were found on the same chromosome have functional similarities and displayed similar responses under metal toxicity. A tandem gene duplication event was detected in GmABC8 and GmABC10.

Ten highly conserved motifs were found in GmABC transporter proteins which belonged to NBDs and TMDs having transporter and ATPase activity. The NBD sequence identity was greater than that of TMDs. All GmABC genes have at least one NBD domain, which indicated that NBD is a unique domain of ABC transporters. Only GmABC6 had unique accessory/regulatory domains related to biotic/abiotic stress tolerance and sulfate transport which determines its role in transporting xenobiotics compounds.

Gene structure revealed that the intron numbers of eight GmABC genes were varied, and only two ABC genes

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(GmABC1 and GmABC8) comprised the same (eight) number of introns. A variable number of introns indicated divergence within GmABC transporters genes.[42] Exon−intron variations were due to the mechanism of intron−exons loss−gain, deletion−insertion, and exonization-pseudo exonization which contributed to gene structural divergence.[49]

Gene ontology described that majority of GmABC proteins were involved in transport, the establishment of localization, nucleotide binding, hydrolase activity, and ATPase activity.[43,44] Hierarchical clustering displayed variations between differentially expressed genes in control and heavy metal (Cd and Hg)-treated plants.

A qRT-PCR-based comparative gene expression indicated that all GmABC transporter gene expression level was altered in the presence of Cd and Hg stress. When exposed to Cd, *Glyma.01G008200.1* (GmABC1), *Glyma.02G038800.1* (GmABC2), *Glyma.05G145000.2* (GmABC3), *Glyma.01G016500.1* (GmABC4), *Glyma.08G070700.1* (GmABC8), *Glyma.05G019400.1* (GmABC9), and *Glyma.08G070800.1* (GmABC10) were significantly upregulated, suggesting their involvement in the transport of heavy metals and the process of detoxification of Cd in soybean plants, this is likely due to the transport and accumulation of phytochelatin-metal (PC−Cd) complexes into the vacuole mediated by these ABC transporters by utilizing ATP and subsequent release of these complexes from the apoplast via internal and external secretion (Figure 10) and supported by some other studies.[7,50]

Similarly, *Arabidopsis* ABC transporter gene, *AtATM3* gene expression was upregulated when exposed to heavy metal cadmium and was implicated in heavy metal tolerance.[44,51] ABC transporter gene *Glyma.08G070700.1* (GmABC8) tends ≥50-fold higher expression in response to Cadmium as compared to control which proposed that it could be highly involved in Cadmium transport and supported by earlier studies in which rice genes, *OsABCG36*, and *OsABCG43* were highly overexpressed and found to be involved in Cadmium resistance.[12,44,52−54] When exposed to Hg stress, *Glyma.01G016500.1* (GmABC4), *Glyma.15G011800.1* (GmABC6), *Glyma.08G070700.1* (GmABC8), and *Glyma.08G070800.1* (GmABC10) genes exhibited certain upregulation indicating their role in Hg transport and tolerance which is likely due to the PC−Hg complex formation in the cytosol and their subsequent transport to plant vacuole through these transporters. These toxic metal complexes were then released from the apoplast. As compared to other genes *Glyma.15G011800.1* (GmABC6) was found to be a potential Hg transporter gene that showed a much higher upregulation (>10-fold expression). Higher upregulation of GmABC6 might be a result of their unique domain organization comprising accessory/regulatory domains which might function toward tolerance to Hg stress.[9,55] Other genes GmABC (1, 2, 3, 5, 7, and 9) were found to be downregulated in response to Hg which pointed out that they may be involved in only Cd transport or another substrates transport activity of plants. The possible mechanism of transport of heavy metals in plants cell through studied ABC transporter genes and their detoxification process is shown in Figure 10.

In response to both Cd and Hg stress, *Glyma.16G172400.5* (GmABC5) and *Glyma.13G002500.1* (GmABC7) genes showed downregulated expression over time, suggesting that these transporter genes may operate in other signal transduction pathways of translocation in soybean plants instead of heavy metal transportation. Genes like GmABC4, GmABC8, and GmABC10 were upregulated in both Cd- and Hg-treated plants, implicating their roles in the transportation of Cd and Hg into the vacuole. Our findings are in agreement with previous studies in other plants which described *AtABCC1*, *AtABCC2*, and *AtABCG36* have been overexpressed in the presence of Cd and Hg.[10,44] Similarly, in other studies, the ABC transporter gene of MDR/ABCB, MRP/ABCC, and PDR/ABCG subfamilies could increase their expression level when exposed to heavy metals Cd and Hg,[11,56] suggesting their role in the detoxification of heavy metals.[57]

Phylogenetic assessment in the present study revealed that ABC transporter proteins from different plant species are being separated by a high bootstrap value (99%).[50,58] These results are in agreement with previous reports.[59] The soybean ABC transporters diverged from the transporters of other plants which suggested that they derived from different ancestral genes.[60−62] Soybean plants underwent whole-genome duplication (WGD) due to which most of the GmABCs were seen clustered into different clades suggesting that ABC transporter gene families in soybean were more diverged and expanded their members over time as a result of segmental and tandem duplications.[50,54,58,63,64]

A few earlier studies have shown that ABC has the largest subfamily and always expands more than others for better adaptation for plants living in altered environments.[57,65] In the phylogenetic tree diagram *Glyma.15G011800.1* (GmABC6) grouped with ABC transporter genes of *A. thaliana* and *V. vinifera* suggested mercury transport and tolerance which has been observed in earlier studies in several plant species.[57,59,66] Our findings revealed that ABC gene family members which were located closely in the phylogenetic shared functional similarities.[67]

Grouping of *Glyma.08G070700.1* (GmABC8) with *Glyma.08G070800.1* (GmABC10) and *Glyma.02G038800.1* (GmABC2) with *Glyma.05G019400.1* (GmABC9) indicated their functional likeness and similar response toward Cd and Hg stress.[54,67] *Glyma.02G038800.1* (GmABC2) and *Glyma.05G019400.1* (GmABC9) grouped with ABC genes of *M. pruriens* and *Z. mays* suggested tolerance to Cadmium. A Cd-responding cluster of multidrug resistance protein family was found comprising *Glyma.01G016500.1* (GmABC4) which was clustered with *Arabidopsis* gene *ATABCB1* which conferred toward Cd resistance.[52,54] *Glyma.01G008200.1* (GmABC1) is related to transporter genes of *T. cacao* and *V. vinifera* which might indicate Cd tolerance.[10,52,68] The significance of doing this phylogenetic analysis is to interpret the relationship of soybean ABC transporter genes with other plants ABC transporters just to check the expression of other related genes in future under stress conditions.

CONCLUSIONS

Taken together, the data we obtained in the present work provide novel information about the altered physiological and biochemical traits and the response of some ABC transporter genes of soybean under Cd and Hg stress. The ABC transporter gene expression of MDR/ABCB, MRP/ABCC, and PDR/ABCG subfamilies was mostly increased under Cd and Hg stress. Sequence information, gene structures, intron-exon pattern, gene ontology, conserved motifs, domain analysis, and hierarchical clustering of ABCs provide a better understanding of ABC transporters' organization and functions. GmABC8 and GmABC6 were identified as potential

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genes having unique domains and exhibiting the highest upregulation which suggests their involvement in Cd and Hg transport, respectively. Significant upregulation in most of the GmABC transporter gene expression patterns in response to Cd and Hg might contribute toward future studies on breeding under stressful conditions. Furthermore, phylogenetic studies provided information for a better understanding of the diversity of soybean ABC transporter genes as well as predicted the evolutionary relationships of soybean ABC transporter genes with other plants' ABC genes.

■ ASSOCIATED CONTENT

Data Availability Statement

Soybean ABC transporter and other plants' gene sequences, protein sequences information and characteristic features, and domain functional analysis were available online in the NCBI, SoyKb (https://soykb.org) and Phytozome v12.1 database (https://phytozome.jgi.doe.gov/pz/portal.html). The software used for Gene identification and functional analysis are MEME 5.4.1 server (https://meme-suite.org); Phyre2 server (http://www.sbg.bio.ic.ac.uk > phyre2); Gene annotation by Gene Ontology Resource (http://geneontology.org); and MetaboAnalyst 3.0 (https://www.metaboanalyst.ca) software for multivariate analysis.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03325.

Mapping of ABC transporter genes in soybean 20 chromosomes in MB-Megabase (0−60); domain organization of ABC transporter proteins in soybean; intron−exon configuration of ABC transporter genes in soybean obtained through Gene Structure Display Server 2.0; GO annotation performed for ABC transporter genes in soybean; full gel view with Real-Time PCR product of selective ABC transporters; primers used for qRT-PCR amplification of ABC transporter genes in soybean and their amplification products; bioinformatical identification of ABC transporter genes from different plant species; sequence information of soybean ABC transporter genes retrieved from the SoyKB database; characteristic properties and subcellular prediction of ABC transporter genes in *G. max*; and domain information of soybean ABC transporter proteins and their functional description obtained through SoyKB and Phytozome server and NCBI (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Rees, D. C.; Johnson, E.; Lewinson, O. ABC transporters: The power to change. *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 218−227.

(2) Gong, X.; Wang, S. New Insights into Evolution of the ABC Transporter Family in Mesostigma viride, a Unicellular Charophyte Algae. *Curr. Issues Mol. Biol.* **2022**, *44*, 1646−1660.

(3) Verrier, P. J.; Bird, D.; Burla, B.; Dassa, E.; Forestier, C.; Geisler, M.; Klein, M.; Kolukisaoglu, U.; Lee, Y.; Martinoia, E.; Murphy, A.; Rea, P. A.; Samuels, L.; Schulz, B.; Spalding, E. P.; Yazaki, K.; Theodoulou, F. L. Plant ABC proteins-a unified nomenclature and updated inventory. *Trends Plant Sci.* **2008**, *13*, 151−159.

(4) Hwang, J. U.; Song, W. Y.; Hong, D.; Ko, D.; Yamaoka, Y.; Jang, S.; Yim, S.; Lee, E.; Khare, D.; Kim, K.; Palmgren, M.; Yoon, H. S.; Martinoia, E.; Lee, Y. Plant ABC Transporters Enable Many Unique Aspects of a Terrestrial Plant's Lifestyle. *Mol. Plant* **2016**, *9*, 338−355.

(5) Martinoia, E.; Massonneau, A.; Frangne, N. Transport Processes of Solutes across the vacuolar membrane of Higher Plants. *Plant Cell Physiol.* **2000**, *41*, 1175−1186.

(6) Kretzschmar, T.; Burla, B.; Lee, Y.; Martinoia, E.; Nagy, R. Functions of ABC transporters in plants. *Essays Biochem.* **2011**, *50*, 145−16060.

(7) Song, W. Y.; Mendoza-Cózatl, D. G.; Lee, Y.; Schroeder, J. I.; Ahn, S. N.; Lee, H. S.; Wicker, T.; Martinoia, E. Phytochelatin-metal (loid) transport into vacuoles shows different substrate preferences in Barley and Arabidopsis. *Plant Cell Environ.* **2014**, *37*, 1192−1201.

(8) Dhara, A.; Raichaudhuri, A. ABCG transporter proteins with beneficial activity on plants. *Phytochem* **2021**, *184*, 112663.

(9) Huang, J.; Li, X.; Chen, X.; Guo, Y.; Liang, W.; Wang, H. Genome-Wide Identification of Soybean ABC Transporters Relate to Aluminum Toxicity. *Int. J. Mol. Sci.* **2021**, *22*, 6556.

(10) Song, W. Y.; Park, J.; Mendoza-Cozatl, D.; Suter-Grotemeyer, M.; Shim, D.; Hortensteiner, S.; Geisler, M.; Weder, B.; Rea, P.; Rentsch, D.; Schroeder, J. I.; Lee, Y.; Martinoia, E. Arsenic tolerance

in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. *Proc. Natl. Acad. Sci.* **2010**, *107*, 21187−21192.
(11) Kang, J.; Park, J.; Choi, H.; Burla, B.; Kretzschmar, T.; Lee, Y.; Martinoia, E. Plant ABC Transporters. *Arabidopsis Book* **2011**, *9*, No. e0153.
(12) Kim, D. Y.; Bovet, L.; Maeshima, M.; Martinoia, E.; Lee, Y. The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. *Plant J.* **2007**, *50*, 207−218.
(13) Dubey, S.; Shri, M.; Gupta, A.; Rani, V.; Chakrabarty, D. Toxicity and detoxification of heavy metals during plant growth and metabolism. *Environ. Chem. Lett.* **2018**, *16*, 1169−1192.
(14) Bagheri, R.; Bashir, H.; Ahmad, J.; Baig, A. M.; Qureshi, M. I. Effects of cadmium stress on plants. *Environ. Sustain. Concepts Princ. Evidences Innov.* **2014**, *7*, 271−277.
(15) Baig, M. A.; Ahmad, J.; Bagheri, R.; Ali, A. A.; Al-Huqail, A. A.; Ibrahim, M. M.; Qureshi, M. I. Proteomic and ecophysiological responses of soybean (Glycine max L.) root nodules to Pb and hg stress. *BMC Plant Biol.* **2018**, *18*, 283.
(16) Turull, M.; Fontàs, C.; Diez, S. Diffusive gradient in thin films with open and restricted gels for predicting mercury uptake by plants. *Environ. Chem. Lett.* **2019**, *17*, 1353−1358.
(17) Stein, M.; Dittgen, J.; Sánchez-Rodríguez, C.; Hou, B.-H.; Molina, A.; Schulze-Lefert, P.; Lipka, V.; Somerville, S. Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. *Plant Cell* **2006**, *18*, 731−746.
(18) Dahuja, A.; Kumar, R. R.; Sakhare, A.; Watts, A.; Singh, B.; Goswami, S.; Sachdev, A.; Praveen, S. Role of ATP-binding cassette transporters in maintaining plant homeostasis under abiotic and biotic stresses. *Physiol. Plant.* **2021**, *171*, 785−801.
(19) Yang, Y. H.; Wang, C. J.; Li, R. F.; Yi, Y. J.; Zeng, L.; Yang, H.; Zhang, C. F.; Song, K. Y.; Guo, S. J. Transcriptome-based identification and expression characterization of RgABCC transporters in Rehmannia glutinosa. *PLoS One* **2021**, *16*, No. e0253188.
(20) Messina, M. Insights gained from 20 Years of Soy Research. *J. Nutr.* **2010**, *140*, 2289−2295.
(21) Clemens, S.; Ma, J. F. Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annu. Rev. Plant Biol.* **2016**, *67*, 489−512.
(22) Xiao, Y.; Du, Y.; Xiao, Y.; Zhang, X.; Wu, J.; Yang, G.; He, Y.; Zhou, Y.; Peijnenburg, W. J. G. M.; Luo, L. Elucidating the effects of TiO_2 nanoparticles on the toxicity and accumulation of Cu in soybean plants (Glycine max L.). *Ecotoxicol. Environ. Saf.* **2021**, *219*, 112312.
(23) Sobariu, D. L.; Fertu, D. I. T.; Diaconu, M.; Pavel, L. V.; Hlihor, R. M.; Dragoi, E. N.; Curteanu, S.; Lenz, M.; Corvini, P. F.; Gavrilescu, M. Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. *N. Biotechnol.* **2017**, *39*, 125−134.
(24) Patra, M.; Sharma, A. Mercury Toxicity in Plants. *J. Stored Prod. Res.* **2000**, *66*, 379−422.
(25) Haider, F. U.; Liqun, C.; Coulter, J. A.; Cheema, S. A.; Wu, J.; Zhang, R.; Wenjun, M.; Farooq, M. Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicol. Environ. Saf.* **2021**, *211*, 111887.
(26) Gill, S. S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909−930.
(27) Ciobanu, C.; Slencu, B. G.; Cuciureanu, R. Estimation of dietary intake of cadmium and lead through food consumption. *Rev. Med.-Chir. Soc. Med. Nat. Iasi* **2012**, *116*, 617−623.
(28) Mobin, M.; Khan, N. A. Photosynthetic activity, pigment composition and antioxidative response of two mustard (Brassica juncea) cultivars differing in photosynthetic capacity subjected to cadmium stress. *J. Plant Physiol.* **2007**, *164*, 601−610.
(29) Zhou, Z. S.; Huang, S. Q.; Guo, K.; Mehta, S. K.; Zhang, P. C.; Yang, Z. M. Metabolic adaptations to mercury-induced oxidative stress in roots of Medicago sativa L. *J. Inorg. Biochem.* **2007**, *101*, 1−9.
(30) Bagheri, R.; Bashir, H.; Ahmad, J.; Iqbal, M.; Qureshi, M. I. Spinach (Spinacia oleracea L.) modulates its proteome differentially in response to salinity, cadmium and their combination stress. *Plant Physiol. Biochem.* **2015**, *97*, 235−245.
(31) Ikhajiagbe, B.; Ogwu, M. C.; Lato, N. F. Growth and yield responses of soybean (Glycine max [L.] Merr.). accessions after exposure to cadmium. *Vegetos* **2021**, *34*, 107−118.
(32) Lone, M. I.; He, Z. L.; Stoffella, P. J.; Yang, X. E. Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. *J. Zhejiang Univ. - Sci. B Biomed. Biotechnol.* **2008**, *9*, 210−220.
(33) Finn, R. D.; Coggill, P.; Eberhardt, R. Y.; Eddy, S. R.; Mistry, J.; Mitchell, A. L.; Potter, S. C.; Punta, M.; Qureshi, M.; Sangrador-Vegas, A.; Salazar, G. A.; Tate, J.; Bateman, A. The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Res.* **2016**, *44*, 279−285.
(34) Goodstein, D. M.; Shu, S.; Howson, R.; Neupane, R.; Hayes, R. D.; Fazo, J.; Mitros, T.; Dirks, W.; Hellsten, U.; Putnam, N.; Rokhsar, D. S. Phytozome: acomparative platform for green plant genomics. *Nucleic Acids Res.* **2012**, *40*, D1178−D1186.
(35) Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, M. R.; Appel, R. D.; Bairoch, A. Protein identification and analysis tools on the expasyserver. In *Proteomics Protocols Handbook*; Walker, J. M., Ed.; Humana Press: Totowa, NJ, 2005; pp 571−607.
(36) Feng, T.; He, X.; Zhuo, R.; Qiao, G.; Han, X.; Qiu, W.; Chi, L.; Zhang, D.; Liu, M. Identification and functional characterization of ABCC transporters for Cd tolerance and accumulation in Sedum alfredii Hance. *Sci. Rep.* **2020**, *10*, 20928.
(37) Bailey, T. L.; Boden, M.; Buske, F. A.; Frith, M.; Grant, C. E.; Clementi, L.; Ren, J.; Li, W. W.; Noble, W. S. MEME SUITE Tools for motif discovery and searching. *Nucleic Acids Res.* **2009**, *37*, 202−208.
(38) Hu, B.; Jin, J.; Guo, A. Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* **2015**, *31*, 1296−1297.
(39) Du, Z.; Zhou, X.; Ling, Y.; Zhang, Z.; Su, Z. Agri GO: a GO analysis toolkit for the agricultural community. *Nucleic Acids Res.* **2010**, *38*, 64−70.
(40) Higgins, D. G.; Thompson, J. D.; Gibson, T. J. Using CLUSTAL for multiple sequence alignments. *Methods in Enzymology*; Elsevier, 1996; Vol. *266*, pp 383−402.
(41) Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. MEGA5:molecular, evolutionary, genetics, analysis, using maximum, likelihood, evolutionary, distance, and maximum, parsimony, methods. *Mol. Biol. Evol.* **2011**, *28*, 2731−2739.
(42) Kim, I. S.; Kim, C. H.; Yang, W. S. Physiologically Active Molecules and Functional Properties of Soybeans in Human Health-A Current Perspective. *Int. J. Mol. Sci.* **2021**, *22*, 4054.
(43) Hanikenne, M.; Motte, P.; Wu, M. C. S.; Wang, T.; Loppes, R.; Matagne, R. F. A mitochondrial halfsize ABC transporter is involved in cadmium tolerance in Chlamydomonas reinhardtii. *Plant Cell Environ.* **2005**, *28*, 863−873.
(44) Park, J.; Song, W. Y.; Ko, D.; Eom, Y.; Hansen, T. H.; Schiller, M.; Lee, T. G.; Martinoia, E.; Lee, Y. The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. *Plant J.* **2012**, *69*, 278−288.
(45) Sánchez-Fernández, R.; Davies, T. G.; Coleman, J. O.; Rea, P. A. The Arabidopsis thaliana ABC Protein Superfamily, a Complete Inventory. *J. Biol. Chem.* **2001**, *276*, 30231−30244.
(46) Lubelski, J.; Konings, W. N.; Driessen, A. J. Distribution and physiology of ABC-type transporters contributing to multidrug resistance in bacteria. *Microbiol. Mol. Biol. Rev.* **2007**, *71*, 463−476.
(47) Rea, P. A. Plant ATP-binding cassette transporters. *Annu. Rev. Plant Biol.* **2007**, *58*, 347−375.
(48) Kelley, L. A.; Mezulis, S.; Yates, C. M.; Wass, M. N.; Sternberg, M. J. E. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* **2015**, *10*, 845−858.
(49) González, L.; González-Vilar, M. Determination of Relative Water Content. In *Handbook of Plant Ecophysiology Techniques*; Springer: Dordrecht, 2001; pp 207−212.

https://doi.org/10.1021/acsomega.3c03325
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(50) Feng, K.; Yu, J.; Cheng, Y.; Ruan, M.; Wang, R.; Ye, Q.; Zhou, G.; Li, Z.; Yao, Z.; Yang, Y.; Zheng, Q.; Wan, H. The SOD Gene Family in Tomato: Identification, Phylogenetic Relationships, and Expression Patterns. *Front. Plant Sci.* **2016**, *7*, 1279.
(51) Eticha, D.; Zahn, M.; Bremer, M.; Yang, Z.; Rangel, A.; Rao, I. M.; Horst, W. J. Transcriptomic analysis reveals differential gene expression in response to aluminium in common bean (Phaseolus vulgaris) genotypes. *Ann. Bot.* **2010**, *105*, 1119−1128.
(52) Ducos, E.; Fraysse, S.; Boutry, M. NtPDR3, an iron-deficiency inducible ABC transporter in Nicotiana tabacum. *FEBS Lett.* **2005**, *579*, 6791−6795.
(53) 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*, 22−32.
(54) 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. *Comput. Biol. Chem.* **2015**, *54*, 18−32.
(55) Singh, V. K.; Jain, M.; Garg, R. Genome-wide analysis and expression profiling reveals diverse roles of GH3 gene family during development and abiotic stress responses in legumes. *Front. Plant Sci.* **2015**, *5*, 789.
(56) Bauer, B. E.; Wolfger, H.; Kuchler, K. Inventory and function of yeast ABC proteins: about sex, stress, pleiotropic drug and heavy metal resistance. *Biochim. Biophys. Acta* **1999**, *1461*, 217−23636.
(57) Lewinson, O.; Livnat-Levanon, N. Mechanism of Action of ABC Importers: Conservation, Divergence, and Physiological Adaptations. *J. Mol. Biol.* **2017**, *429*, 606−619.
(58) Lopez-Ortiz, C.; Dutta, S. K.; Natarajan, P.; Peña-Garcia, Y.; Abburi, V.; Saminathan, T.; Nimmakayala, P.; Reddy, U. K. Genome-wide identification and gene expression pattern of ABC transporter gene family in Capsicum spp. *PLoS One* **2019**, *14*, No. e0215901.
(59) 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. *Front. Plant Sci.* **2017**, *8*, 349.
(60) Kovalchuk, A.; Driessen, A. J. Phylogenetic analysis of fungal ABC transporters. *BMC Genom.* **2010**, *11*, 177.
(61) 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.
(62) Lane, T. S.; Rempe, C. S.; Davitt, J.; Staton, M. E.; Peng, Y.; Soltis, D. E.; Melkonian, M.; Deyholos, M.; Leebens-Mack, J. H.; Chase, M.; et al. Diversity of ABC transporter genes across the plant kingdom and their potential utility in biotechnology. *BMC Biotechnol.* **2016**, *16*, 47.
(63) Andolfo, G.; Ruocco, M.; Di Donato, A.; Frusciante, L.; Lorito, M.; Scala, F.; Ercolano, M. R. Genetic variability and evolutionary diversification of membrane ABC transporters in plants. *BMC Plant Biol.* **2015**, *15*, 51.
(64) Zhang, Z.; Tong, T.; Fang, Y.; Zheng, J.; Zhang, X.; Niu, C.; Li, J.; Zhang, X.; Xue, D. Genome-wide identification of Barley ABC genes and their expression in response to abiotic stress treatment. *Plants* **2020**, *9*, 1281.
(65) Davies, T. G.; Coleman, J. O. D. The Arabidopsis thaliana ATP-binding cassette proteins: An emerging superfamily. *Plant Cell Environ.* **2000**, *23*, 431−443.
(66) Nguyen, V. N. T.; Moon, S.; Jung, K. H. Genome-wide expression analysis of rice ABC transporter family across spatio-temporal samples and in response to abiotic stresses. *J. Plant Physiol.* **2014**, *171*, 1276−1288.
(67) Dassa, E.; Bouige, P. The ABC of ABCs: a phylogenetic and functional classification of ABC systems in living organisms. *Res. Microbiol.* **2001**, *152*, 211−229.
(68) Fu, S.; Lu, Y.; Zhang, X.; Yang, G.; Chao, D.; Wang, Z.; Shi, M.; Chen, J.; Chao, D. Y.; Li, R.; Ma, J. F.; Xia, J. The ABC transporter ABCG36 is required for cadmium tolerance in rice. *J. Exp. Bot.* **2019**, *70*, 5909−5918.

https://doi.org/10.1021/acsomega.3c03325
ACS Omega 2023, 8, 27597−27611