Transcriptome-wide identification and expression analyses of ABC transporters in dwarf polish wheat under metal stresses

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Abstract

ABC transporters, which comprise one of the largest protein families, are involved in maintaining osmotic homeostasis, nutrient uptake, pathogen resistance, and metal tolerance. In this study, 30 *ABC* genes in dwarf polish wheat were characterized and classified into seven subfamilies (ABCA - ABCG). Among them, 24 ABC transporters were newly found in wheat. The expressions of 13 *ABC* genes in roots and leaves under six metal stresses were also analyzed. All these genes were differentially regulated by Cd (except *ABCE2*, *ABCF4*, and *ABCF6* in roots), suggesting that these genes participate in Cd transport, sequestration, or uptake. These genes were also differentially regulated by other metals including Cu, Mg, Zn, Fe, and Ni. Results suggest that the expressions of ABC transporters in dwarf polish wheat played important roles in metal transport and detoxification.

Additional key words: cadmium, copper, gene expression, iron, magnesium, nickel, zinc.

Introduction

Metals are classified into essential and non-essential elements. Essential metals, including Mg, Fe, Cu, Zn, and Ni, play important roles in plant metabolism, development, and growth (Hansch and Mendel 2009, Waraich *et al.* 2011). However, some non-essential elements, such as Cd, significantly inhibit plant growth and development (Jarup 2003). Normally, these metals coexist in soil. Therefore, plant metal transporters are involved in metal uptake, transport, and sequestration to prevent damage from non-essential metals and ensure appropriate homeostasis of essential minerals (Lin and Aarts 2012). For example, the ATP-binding cassette (ABC) transporter family, which is one of the largest transporter families, enhances metal tolerance and metal accumulation in plants (Henikoff *et al.* 1997, Song *et al.* 2010, Park *et al.* 2012).

At present, 48 ABC transporters have been identified in humans (Dean *et al.* 2001), 31 in yeast (Rajendra and Andre 2012), and over 80 in *Escherichia coli* (Blattner *et al.* 1997). In plants, 131 ABC transporters have been identified in *Arabidopsis* (Sanchez-Fernandez *et al.* 2001, Martinoia *et al.* 2002), 133 in rice (Verrier *et al.* 2008, Nguyen *et al.* 2014), and 130 in maize (Pang *et al.* 2013). All these ABC transporters are classified into eight subfamilies (ABCA - ABCI, ABCH was not found in plants) (Verrier *et al.* 2008) and play different roles in diverse cellular processes, such as osmotic homeostasis (Ames *et al.* 1990), nutrient uptake (Martinoia *et al.* 2002), hormone transport (Kuromori *et al.* 2010), fatty acid import (Zolman *et al.* 2001), pathogen resistance (Krattinger *et al.* 2009, 2011), and metal tolerance (Moons 2003, Bovet *et al.* 2005, Kim *et al.* 2007, Park *et al.* 2012). To date, only a few ABC transporters have been identified and characterized in wheat. *TaMDR1* participates in the early metabolic disorders of calcium ion in the root apex under Al stress (Takayuki *et al.* 2002). *TaMRP1* performs a potential role in glutathione-mediated detoxification (Theodoulou *et al.* 2003). *Lr34* plays crucial roles in rust resistance in wheat (Krattinger *et al.* 2009, 2011). *TaPDR1* and *TaABCG2-6* enhance

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the resistance to fusarium head blight (Shang *et al.* 2009, Muhovski *et al.* 2014). Recently, 18 *ABCC* genes in wheat were observed. They are expressed in different tissues; their transcripts are differentially regulated by Cd, hormones, and H_2O_2; one of them, TaABCC13, participates in glutathione-mediated detoxification pathway (Bhati *et al.* 2015); other two *TaABCC3* genes contribute to mycotoxin deoxyni-valenol tolerance, grain formation and ripening (Walter *et al.* 2015). Since the genome size of wheat is bigger than that of *Arabidopsis*, rice, and maize, 130 or more ABC transporters should exist in wheat. Observation of more ABC transporters from wheat and their responses to metals are interesting and important for wheat improvement.

Moreover, there is no information on ABC transporters from tetraploid wheat. As one of the important cultivar-groups, polish wheat (2n = 4x = 28, AABB, *Triticum polonicum* L.) has low genetic similarity to *T. durum*, *T. turgidum*, and *T. aestivum* (Wang *et al.* 2013, Michalcová *et al.* 2014), and accumulate high content of Zn in seeds (Wiwart *et al.* 2013). Meanwhile, in the present study, dwarf polish wheat (DPW, *T. polonicum*) also accumulates high concentrations of Cd and Zn in its roots or shoots, and exhibits high Cd and Zn tolerances. Therefore, it is interesting to identify and characterize ABC transporters responsible for processes caused by metal stresses, which could illustrate the mechanisms of high tolerance to Cd and Zn and other metals in DPW.

Materials and methods

Characterization of ABC transporters: DPW, a spontaneous mutant dwarf accession of *Triticum polonicum* L., was originally collected in Tulufan, Xinjiang, China, and maintained at the Triticeae Research Institute, Sichuan Agricultural University, Sichuan, China.

A transcriptome of DPW, including 76 014 unigenes assembled using *Trinity* software (Grabherr *et al.* 2011), was constructed from roots, stems, and leaves using *RNA-Seq* on the platform of *Illumina HiSeq 2000*. Transcriptome data have been deposited at GenBank under the accession GEDT00000000. 81.23 % unigenes matched at least one functionally annotated gene in at least one database of the *NCBI NR, NT, Swiss-Prot, KEGG, KOG*, and *COG*, as determined using *BLASTX* with an e-value below e^{-5} (Wang *et al.* 2016). On the basis of the transcriptome of DPW, 30 functionally annotated ABC genes with complete open reading frames (ORFs) were found (GenBank accession numbers are listed in Table 1 Suppl.). These 30 ABC transporters from DPW together with other plant ABC transporters, composed of 21 transporters from wheat, 108 from rice, and 106 from *Arabidopsis* (acc. Nos. of all the genes are listed in Table 1 - 3 Suppl.) were used to perform phylogenetic analysis using *MUSCLE* software (Edgar 2004, http://www. drive5.com/muscle) for sequence alignment and *MEGA 5.05* software (Tamura *et al.* 2011, http://www. megasoftware.net) for tree calculation using the neighbour-joining method. Bootstrap analysis (1 000 replicates) confirmed the structure of the tree. The 30 *TpABC* sequences were individually mapped over wheat genome draft sequences using *BLAST* (http://www. gramene.org/).

Plants and growth conditions: The seeds of DPW were sterilized with 5 % (m/v) NaClO for 5 min and washed thrice with double distilled water. After germination for 5 d at room temperature, seedlings were cultured in distilled water (dH$_2$O) for 5 d and then transferred to nutrient solution (Hoagland's modified basal salt mixture, *MP Biomedicals*, CA, USA) in a growth chamber under 16-h photoperiod, an irradiance of 180 μmol m^{-2} s^{-1}, a temperature of 25 °C, and an air humidity of 75 %. After 9 d of culture in nutrient solution, the seedlings were exposed to CdSO$_4$ (40 μM), NiCl$_2$ (40 μM), ZnSO$_4$ (8 mM), MgCl$_2$ (8 mM), FeCl$_3$ (8 mM), and CuCl$_2$ (8mM), respectively. The leaf and root samples were collected from 5 - 8 seedlings at five sampling times (2, 4, 8, 12, and 24 h after treatment) with 3 biological replicates, then snap-frozen in liquid nitrogen and stored at -80 °C for RNA isolation.

Determination of growth, water content, and metal content in roots and leaves: Seedlings from each treatment were harvested after 24 h. Root samples were treated with 0.1 % EDTA solution and then washed twice with dH$_2$O. The length of roots and leaves was the mean value of five longest seedlings of each treatment. The water content (WC) of 30 seedlings was determined using the following formula: WC [%] = [(FM - DM)/FM] × 100 (Kim *et al.* 2005), where FM was fresh mass and DM was dry mass determined after drying at 60 °C for 72 h.

All dried samples were ground to a fine powder and digested in mixed acids [HNO$_3$ + HClO$_4$ (3:1, v/v)] (Ait *et al.* 2004) for determination of metal elements. Content of Mg, Zn, Fe, Cu, Cd, and Ni was determined using an atomic absorption spectrometer (*Shimadzu AA-6300*, Kyoto, Japan).

RNA extraction, cDNA synthesis, primer design, and real time qPCR: Total RNA was extracted using *E.Z.N.A.*® total RNA kit II (*Omega*, Shanghai, China) according to the user manual. RNase-free DNase (*Omega*) was used to remove the genomic DNA. RNA content was measured twice with a *NanoDrop-2000* spectrophotometer (*NanoDrop Technologies*, Wilmington, USA).

Total 2 μg of RNA was used to synthesize cDNA using *M-MLV* first strand cDNA synthesis kit (*Omega*). All the

cDNA samples were diluted 10× for real time qPCR performance. Two genes of each subfamily, totally 13 ABC transporters (ABCE subfamily had one gene), were randomly selected for real time qPCR analysis. All the primers were designed using *Beacon Designer 7* (http://www.premierbiosoft.com/molecular_beacons/index.html) (Table 4 Suppl.).

Real time qPCR was performed in 96-well plates using *CFX-96TM* real-time system (*Bio-Rad*, Hercules, CA, USA). Each reaction contained 6.3 mm^3 of cDNA (10 ng mm^{-3}), 0.6 mm^3 of each primer (3 pmol mm^{-3}), and 7.5 mm^3 of iQ^{TM} *SYBR Green* supermix (*Bio-Rad*), with a total volume of 15 mm^3. A no-template control contained 6.3 mm^3 of RNase free water. All the reactions were carried out in triplicate for each cDNA sample and three technical replicates. *Actin* (*ACT*) (Table 4 Suppl.) was used as reference gene for normalization (Wang *et al.* 2015). The PCR conditions were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 15 s at 95 °C and 1 min at 60 °C, followed by the generation of a dissociation curve by increasing the temperature from 65 to 95 °C to check for amplification specificity (Wang *et al.* 2012, 2015). The baseline and threshold cycles (Ct values) were automatically determined with the default parameters. Relative expressions were calculated using *Bio-Rad CFX Manager v. 1 6.541.1028* using the ΔΔCt method (Livak and Schmittgen 2001). Compared with the control, threefold change or more with $P < 0.01$ (Dunnett's test) were considered significant regulations. To detect the specificity of these primers, the amplifications of 13 genes were cloned and sequenced at *Sangon Biotech* (Shanghai, China).

Results

Thirty ABC transporters with complete ORFs were identified from DPW transcriptome (Fig. 1 Suppl.). These 30 ABC transporters with 24 newly found in wheat were classified into seven subfamilies. All gene names and groups are listed in Table 1 Suppl. Among these 30 ABC transporters, all 6 ABCC transporters TaABCC1,
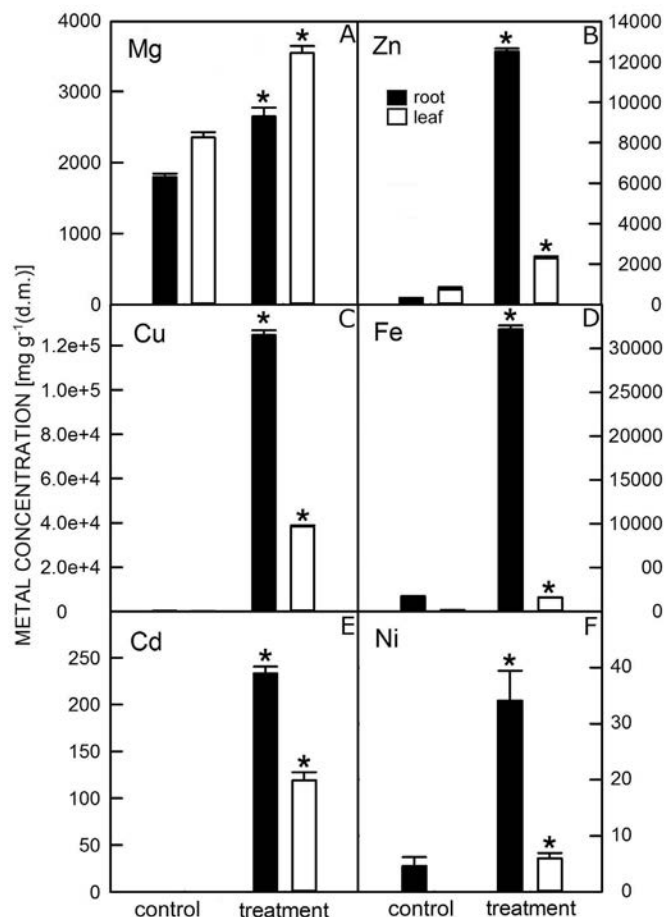

Fig. 1. The metal content after treatment with MgCl$_2$ (8 mM, *A*), ZnSO$_4$ (8 mM, *B*), CuCl$_2$ (8 mM, *C*), FeCl$_3$ (8 mM, *D*), CdSO$_4$ (40 μM, *E*), and NiCl$_2$ (40 μM, *F*) in roots and leaves. Means ± SDs, $n = 3$, * - significant differences at $P \leq 0.05$.

TaABCC3, TaABCC7, TaABCC8, TaABCC9, and TaABCC13 were closely related. Meanwhile, all 30 ABC transporters had Walker A motif (Walker *et al.* 1982, GXXGXGKS/T, X can be varied), signature motif (Higgins 1992, LSGGQKQR), and Walker B motif (Walker *et al.* 1982, hhhhD, "h" stands for hydrophobic)

(Fig. 2 Suppl.). All 30 *TpABC* genes were mapped onto Chinese spring wheat chromosomes using *BLAST* (http://www.gramene.org/) (Table 5 Suppl.) and 29 *TpABC* genes were mapped on all three wheat genomes, except for *TpABCC3* which was mapped on A and B genomes, suggesting the presence of homologous sequences.
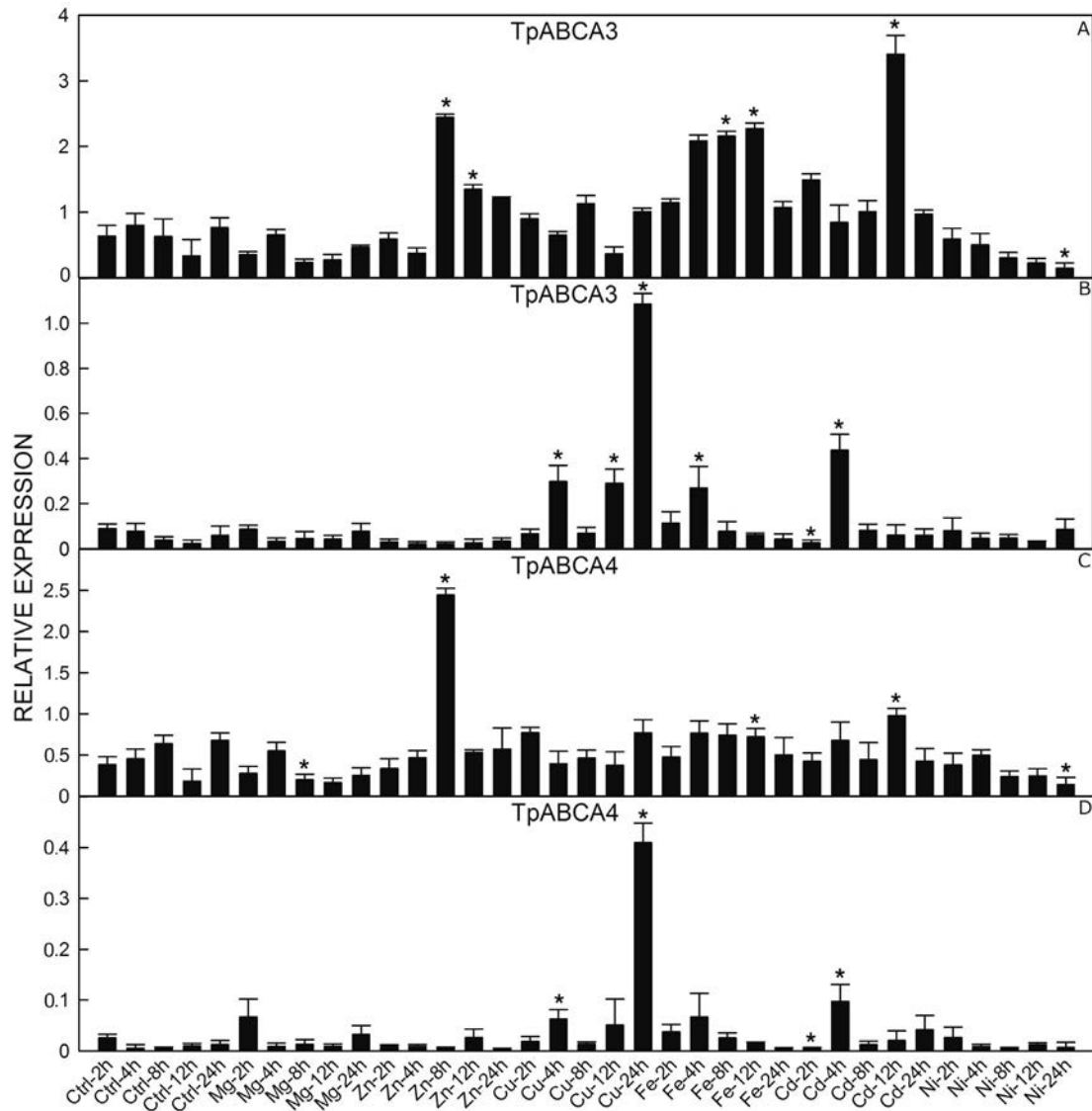

Fig. 2. Relative expressions of *TpABCA3* and *TpABCA4* in the roots (*A, C*) and leaves (*B, D*) under different metal stresses. Means ± SDs, *n* = 3, * - significant regulation > threefold or < 0.33-fold and *P* < 0.01. Ctrl - control.

All metals were taken up and accumulated 24 h after treatments (Fig. 1). The metal content in all treated roots and leaves were significantly higher than in their corresponding controls. Except for Mg (Fig. 1*A*), metal content in leaves was significantly lower than in roots (Fig. 1*B-F*).

Compared with control, Cu significantly inhibited the growth of leaves and the seedlings growth was not affected by other metals (Fig. 3 Suppl.). Similarly, compared with control, all metal did not significantly

change the water content in both roots and leaves (Fig. 3 Suppl.).

Since ABC transporters respond to metals (Moons 2003, Bovet *et al.* 2005, Kim *et al.* 2007, Park *et al.* 2012), and roots and leaves accumulated high metal content under metal stress (Fig. 1), theoretically, the expressions of *TpABC* transporters would be regulated by metals.

Compared with the control, the expression of *TpABCA3* in roots was significantly up-regulated by
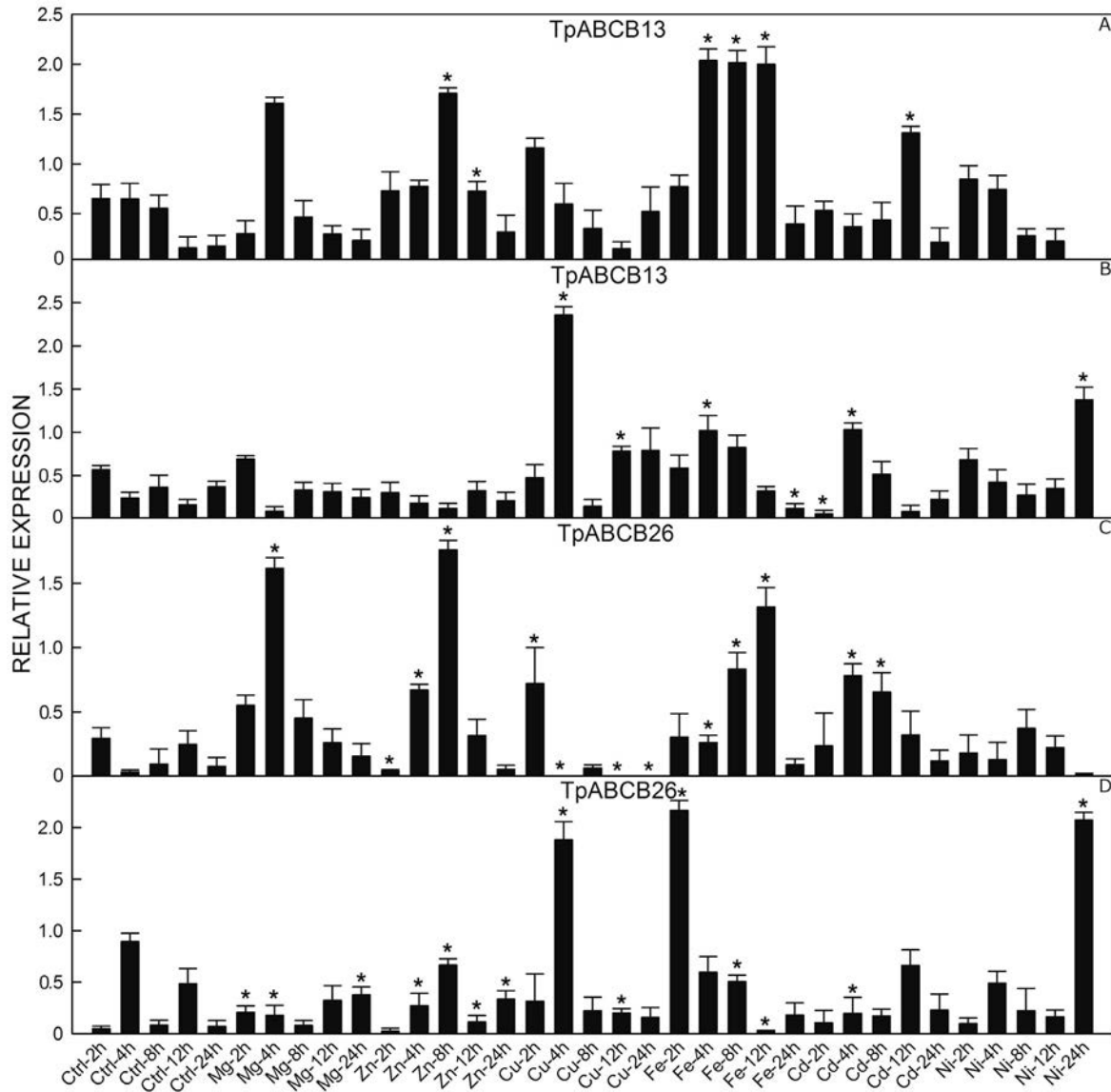
Fig. 3. Relative expressions of *TpABCB13* and *TpABCB26* in the roots (*A, C*) and leaves (*B, D*) under different metal stresses. Means ± SDs, *n* = 3, * - significant regulation > threefold or < 0.33-fold) and *P* < 0.01.

Zn^{2+} at 8 and 12 h, Fe^{3+} at 8 and 12 h, Cd^{2+} at 12 h, and down-regulated by Ni^{2+} at 24 h (Fig. 2*A*). In leaves, *TpABCA3* was significantly up-regulated by Cu^{2+} at 4, 12, and 24 h, Fe^{3+} at 4 h, Cd^{2+} at 4 h, and down-regulated by Cd^{2+} at 2 h (Fig. 2*B*). Compared with the control, the expressions of *TpABCA4* in roots were dramatically up-regulated by Zn^{2+} at 8 h, Fe^{3+} at 12 h, Cd^{2+} at 12 h, and down-regulated by Mg^{2+} at 8 h and Ni^{2+} at 24 h (Fig. 2*C*). In leaves, the expressions of *TpABCA4* were up-regulated by Cu^{2+} at 4 and 24 h, Cd^{2+} at 4 h, and down-regulated by Cd^{2+} at 2 h (Fig. 2*D*).

Compared with the control, the expressions of *TpABCB13* in roots were significantly up-regulated by Zn^{2+} at 8 and 12 h, Fe^{3+} at 4, 8, and 12 h, and Cd^{2+} at 12 h (Fig. 3*A*). In leaves, *TpABCB13* was significantly up-

regulated by Cu^{2+} at 4 and 12 h, Fe^{3+} at 4 h, Cd^{2+} at 4 h, and Ni^{2+} at 24 h, and down-regulated by Fe^{3+} at 24 h, Cd^{2+} at 2 h (Fig. 3*B*). In roots, the expressions of *TpABCB26* were strongly up-regulated by Mg^{2+} at 4 h, Zn^{2+} at 4 and 8 h, Fe^{3+} at 4, 8, and 12 h, and Cd^{2+} at 4 and 8 h, down-regulated by Zn^{2+} at 2 h, and completely repressed by Cu^{2+} after 4, 12, and 24 h (Fig. 3*C*). In leaves, *TpABCB26* was dramatically up-regulated by Mg^{2+} at 2 and 24 h, Zn^{2+} at 8 and 24 h, Fe^{3+} at 2 and 8 h, and Ni^{2+} at 24 h, down-regulated by Mg^{2+} at 4 h, Zn^{2+} at 4 and 12 h, Fe^{3+} at 12 h, Cd^{2+} at 4 h (Fig. 3*D*).

Compared with the control, the expressions of *TpABCC1* in roots were significantly up-regulated by Mg^{2+} at 4 h, Zn^{2+} at 8 h, Fe^{3+} at 2, 8, and 12 h, and Cd^{2+} at 2 h (Fig. 4*A*). Its expressions in leaves were dramatically

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down-regulated by Zn^{2+} at 8 h, Fe^{3+} at 4 h, Cd^{2+} at 8 h, and Ni^{2+} at 8 h and up-regulated by Cu^{2+} at 12 h and Ni^{2+} at 24 h (Fig. 4*B*). Compared with the control, the expressions of *TpABCC9* in roots were evidently up-regulated by Cu^{2+} at 24 h, Fe^{3+} at 12 h, and Cd^{2+} at 12 h

and down-regulated by Mg^{2+} at 2 h (Fig. 4*C*). Its expressions in leaves were significantly up-regulated by Mg^{2+} at 2 h, Cu^{2+} at 4 h, Fe^{3+} at 4 h, and Cd^{2+} at 4 h and down-regulated by Cd^{2+} at 2 h (Fig. 4*D*).
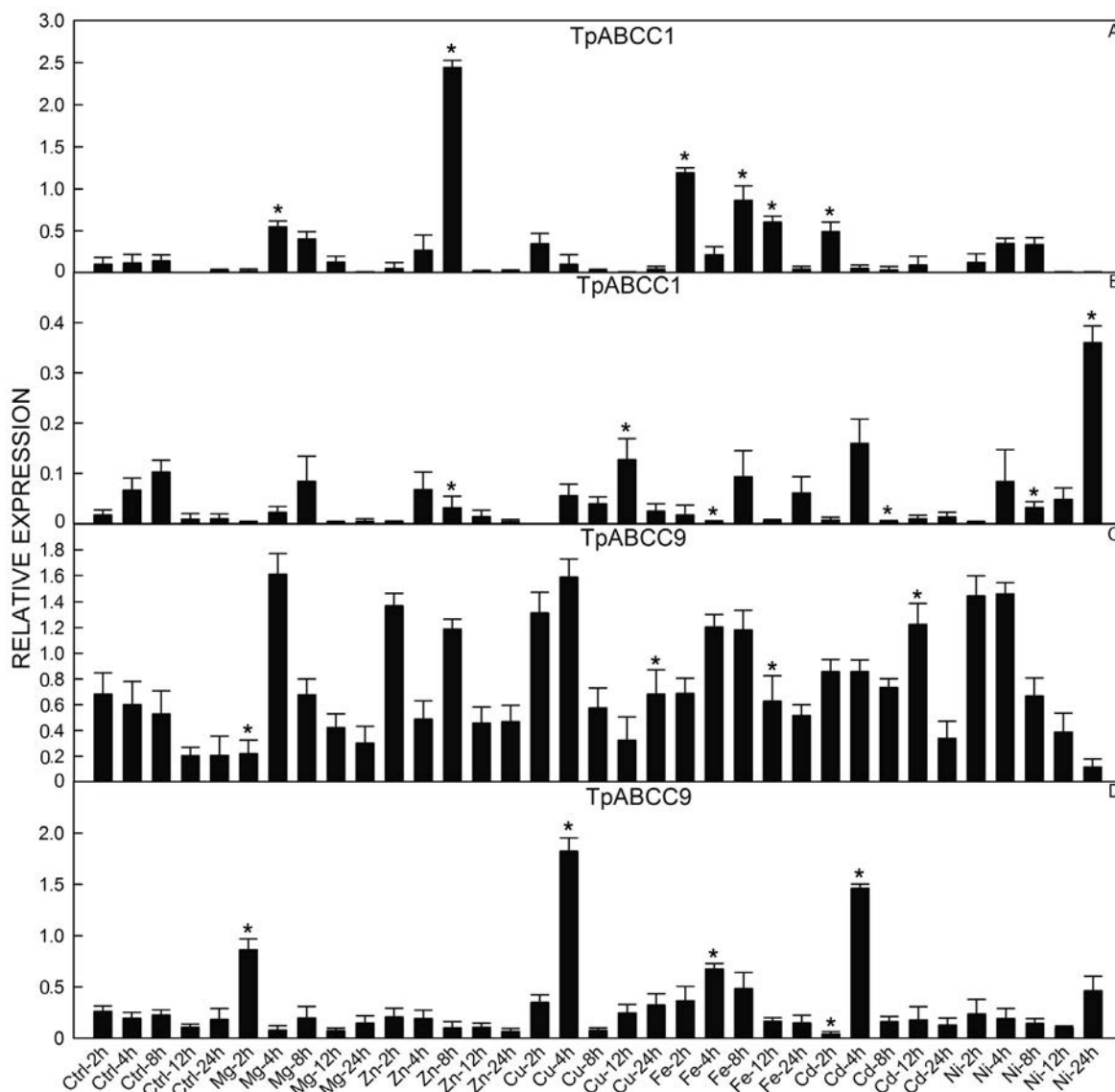

Fig. 4. Relative expressions of *TpABCC1* and *TpABCC9* in the roots (*A, C*) and leaves (*B, D*) under different metal stresses. Means ± SDs, $n = 3$, * - significant regulation > threefold or < 0.33-fold and $P < 0.01$.

Compared with the control, the expressions of *TpABCD1* in roots were obviously up-regulated by Fe^{3+} at 2 h, Cd^{2+} at 2 and 12 h, and Ni^{2+} at 4 h (Fig. 5*A*). In leaves, *TpABCD1* was strongly up-regulated by Cu^{2+} at 4 h, Fe^{3+} at 8 h, and Cd^{2+} at 4 h, and down-regulated by Cd^{2+} at 2 h (Fig. 5*B*). The expressions of *TpABCD2* in roots were lower than in leaves in the control (Fig. 5*C, D*). Compared with the control, the expressions of *TpABCD2* in roots were significantly up-regulated by Mg^{2+} at 4 and 12 h, Zn^{2+} at 4 and 8 h, Cd^{2+} at 12 h, and

Ni^{2+} at 4 and 8 h (Fig. 5*C*). The expressions of *TpABCD2* in leaves were strongly down-regulated by Mg^{2+} at 12 h, Cu^{2+} at 2 h, Fe^{3+} at 12 h, Cd^{2+} at 8 h and up-regulated by Cu^{2+} at 4 h, Cd^{2+} at 4 h, and Ni^{2+} at 24 h (Fig. 5*D*).

Compared with the control, the expressions of *TpABCE2* in roots were evidently down-regulated by Mg^{2+} at 8 and 24 h, Zn^{2+} at 8 h, and Ni^{2+} at 24 h and up-regulated by Mg^{2+} at 12 h, Fe^{3+} at 12 h, and Ni^{2+} at 2 and 4 h (Fig. 6*A*). The expressions in leaves were strongly up-regulated by Cu^{2+} at 4 and 24 h, Cd^{2+} at 4, 12, and 24 h,

and Ni^{2+} at 12 and 24 h and down-regulated by Cd^{2+} at 8 h (Fig. 6B). The expressions of *TpABCF4* in roots were evidently up-regulated by Cu^{2+} at 12 h, Fe^{3+} at 12 h, and Ni^{2+} at 8 h (Fig. 7A). Its expressions in leaves were significantly down-regulated by Mg^{2+} at 12 h, Cd^{2+} at 2 h and up-regulated by Cu^{2+} at 4 h, Fe^{3+} at 4 h, Cd^{2+} at 4 h, and Ni^{2+} at 24 h (Fig. 7B). Compared with the control, the

expressions of *TpABCF6* in roots were significantly up-regulated by Fe^{3+} at 12 h and down-regulated by Mg^{2+} at 24 h, Zn^{2+} at 4 h, and Ni^{2+} at 24 h (Fig. 7C). Its expressions in leaves were strongly up-regulated by Cu^{2+} at 4 h and Cd^{2+} at 4 and 24 h, and down-regulated by Mg^{2+} at 2 and 8 h, Zn^{2+} at 8 h, Cu^{2+} at 12 h, and Cd^{2+} at 2 h (Fig. 7D).
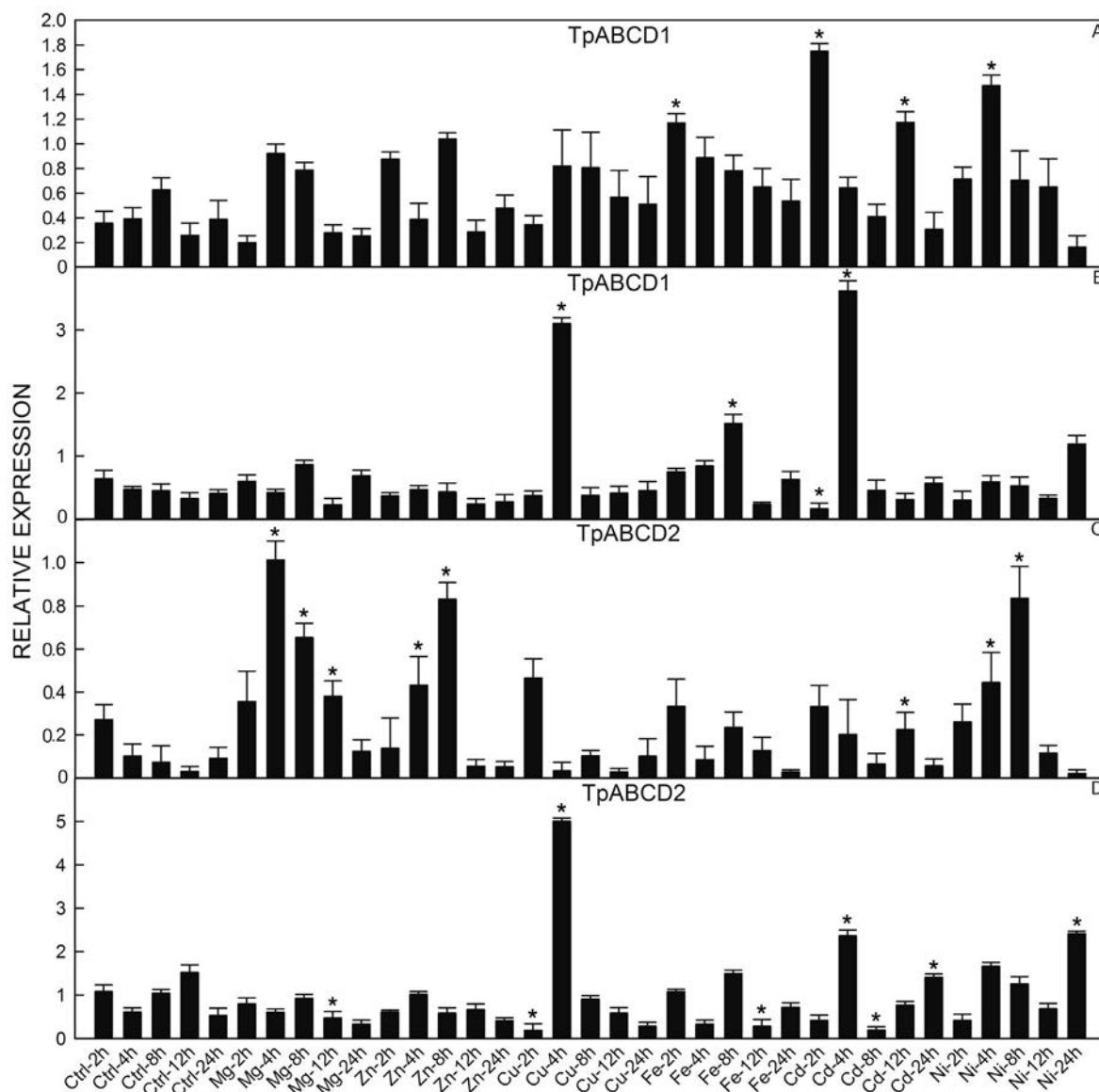

Fig. 5. Relative expressions of *TpABCD1* and *TpABCD2* in the roots (A, C) and leaves (B, D) under different metal stresses. Means ± SDs, $n = 3$, * - significant regulation > threefold or < 0.33-fold and $P < 0.01$.

Compared with the control, the expressions of *TpABCG8* in roots were significantly up-regulated by Zn^{2+} at 12 h, Cu^{2+} at 4, 12, and 24 h, Fe^{3+} at all sampling times, and Cd^{2+} at 12 h, but down-regulated by Ni^{2+} at 24 h (Fig. 8A). In leaves, *TpABCG8* expressions were strongly up-regulated by Cu^{2+} at 4 - 24 h, Fe^{3+} at 4 h, and Cd^{2+} at 4 h (Fig. 8B). In the control, the expressions of *TpABCG42* in roots were lower than in leaves

(Fig. 8C,D). Compared with the control, the expressions of *TpABCG42* in roots were dramatically up-regulated by Mg^{2+} at 2 - 12 h, Zn^{2+} at 4 and 8 h, Cu^{2+} at 2 - 12 h, Fe^{3+} at 2 - 12 h, Cd^{2+} at 2 h, and Ni^{2+} at 2 h and 8 - 24 h (Fig. 8C). The expressions in leaves were strongly up-regulated by Cu^{2+} at 12 h and Cd^{2+} at 2 h, but down-regulated by Mg^{2+} at 12 - 24 h, Zn^{2+} at 12 - 24 h, Cu^{2+} at 24 h, Fe^{3+} at 4 - 12 h, and Cd^{2+} at 4 and 8 h (Fig. 8D).
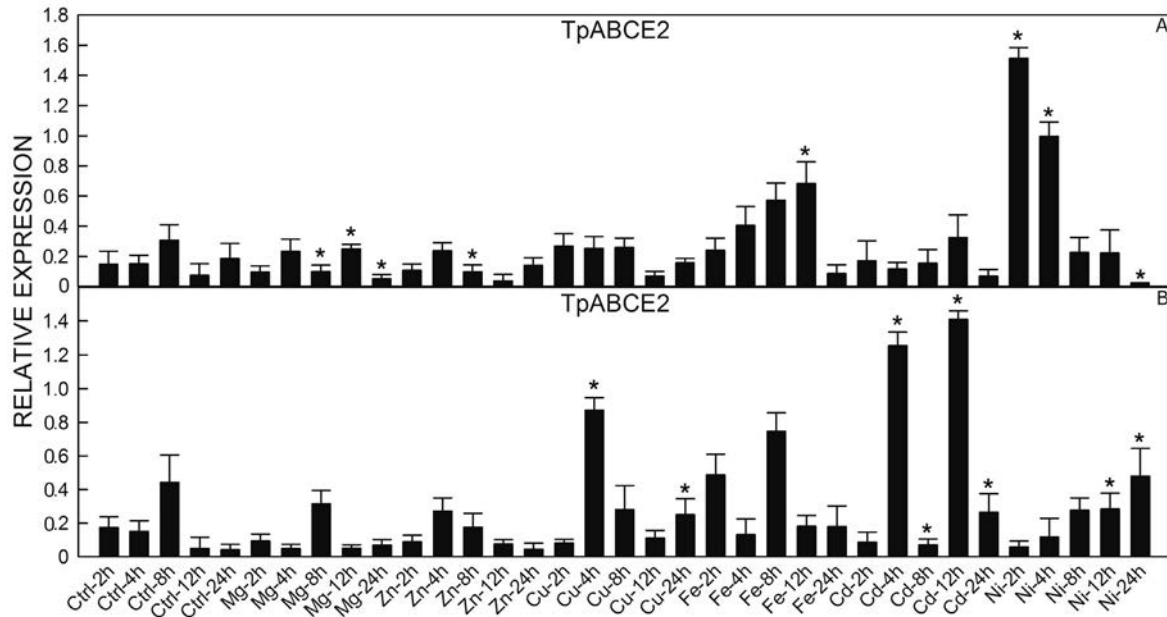
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Fig. 6. Relative expressions of *TpABCE2* in the roots (*A*) and leaves (*B*) under different metal stresses. Means ± SDs, *n* = 3, * - significant regulation >threefold or <0.33-fold and *P* < 0.01.

Discussion

Based on the published genomes, 130 or more ABC transporters in *Arabidopsis*, rice, or maize have been discovered and some of them have been cloned and functionally analyzed (Sanchez-Fernandez *et al.* 2001, Pang *et al.* 2013, Nguyen *et al.* 2014). Only 27 ABC transporters have been discovered in wheat and a few transporters were functionally analyzed (Takayuki *et al.* 2002, Theodoulou *et al.* 2003, Shang *et al.* 2009, Krattinger *et al.* 2009, 2011, Muhovski *et al.* 2014, Bhati *et al.* 2015). Fortunately, *RNA-Seq* were widely used to identify novel or known genes in plants lacking genome information (Duan *et al.* 2012, Schreiber *et al.* 2012). In the present study, 30 ABC transporters were identified from DPW using *RNA-Seq*. Among them, 24 ABC genes were newly found in wheat and six ABCC members were closely related to TaABCC1, TaABCC3, TaABCC7, TaABCC8, TaABCC9 and TaABCC13, respectively. Since 130 or more ABC transporters should exist in wheat, approximately 100 ABC transporters were not identified in tetraploid wheat, which might result from that some ABC transporters produced by *RNA-seq* had small segments or pseudogenes resulting from assembly errors (Sanchez-Fernandez *et al.* 2001, Pang *et al.* 2013) and different genes had specific expression in different tissues, such as some *TaABCC* genes are only expressed in flag leaves or seeds (Bhati *et al.* 2015).

ABC transporters could play important role in the transport of various substances, including ions, lipids, antibiotics, drugs, and heavy metals (Martinoia *et al.* 2002, Kim *et al.* 2007). Several studies have demon-

strated that ABC transporters are involved in metal tolerance and accumulation (Moons 2003, Bovet *et al.* 2005, Kim *et al.* 2007, Park *et al.* 2012). ABC transporters from yeasts or other non-plant species, such as ScYCF1, SpHMT1, CeHMT1, and DmHMT1, sequester Cd into vacuoles to enhance Cd resistance (Ortiz *et al.* 1995, Li *et al.* 1997, Vatamaniuk *et al.* 2005, Prévéral *et al.* 2009, Sooksa-Nguan *et al.* 2009). Moreover, an *ABCC* gene from *Glomus intraradices* is also up-regulated by Cd (González-Guerrero *et al.* 2010). In plants, several ABC transporters are involved in Cd transport or resistance. In *Arabidopsis*, AtABCC1, AtABCC2, AtABCC5, and AtABCG36 are involved in Cd tolerance, but are not regulated by Cd (Kim *et al.* 2007, Kretzschmar *et al.* 2011, Park *et al.* 2012). The over-expression of *AtABCC1* enhances Cd tolerance and accumulation (Park *et al.* 2012). The expressions of *AtATM3* (*AtABCB25*), *AtABCG36*, *AtABCC3*, and *AtABCC6* are significantly up-regulated by Cd (Bovet *et al.* 2003, Kim *et al.* 2006, 2007, Gaillard *et al.* 2008). In rice, the expression of *OsPDR9* (*OsABCG36*) is induced by Cd (Moons 2003). In wheat, the expressions of *TaABCC3*, *TaABCC4*, *TaABCC11*, and *TaABCC14* are up-regulated by Cd, whereas *TaABCC1*, *TaABCC6*, and *TaABCC9* are slightly repressed (Bhati *et al.* 2015). In this study, the expressions of all 13 *ABC* genes were differentially regulated by Cd (except of *ABCE2*, *ABCF4*, and *ABCF6* in roots). The regulations induced by Cd were different from those in other reports, which may result from the varying sampling times and Cd

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concentrations (Bovet *et al.* 2003, Kim *et al.* 2006, 2007, Gaillard *et al.* 2008, Bhati *et al.* 2015).Thus, all the results indicated that these *TpABC* genes might play

different roles in Cd transport, sequestration, or uptake.

Meanwhile, several studies have also indicated that the expressions of ABC genes, such as *OsABCG36*,
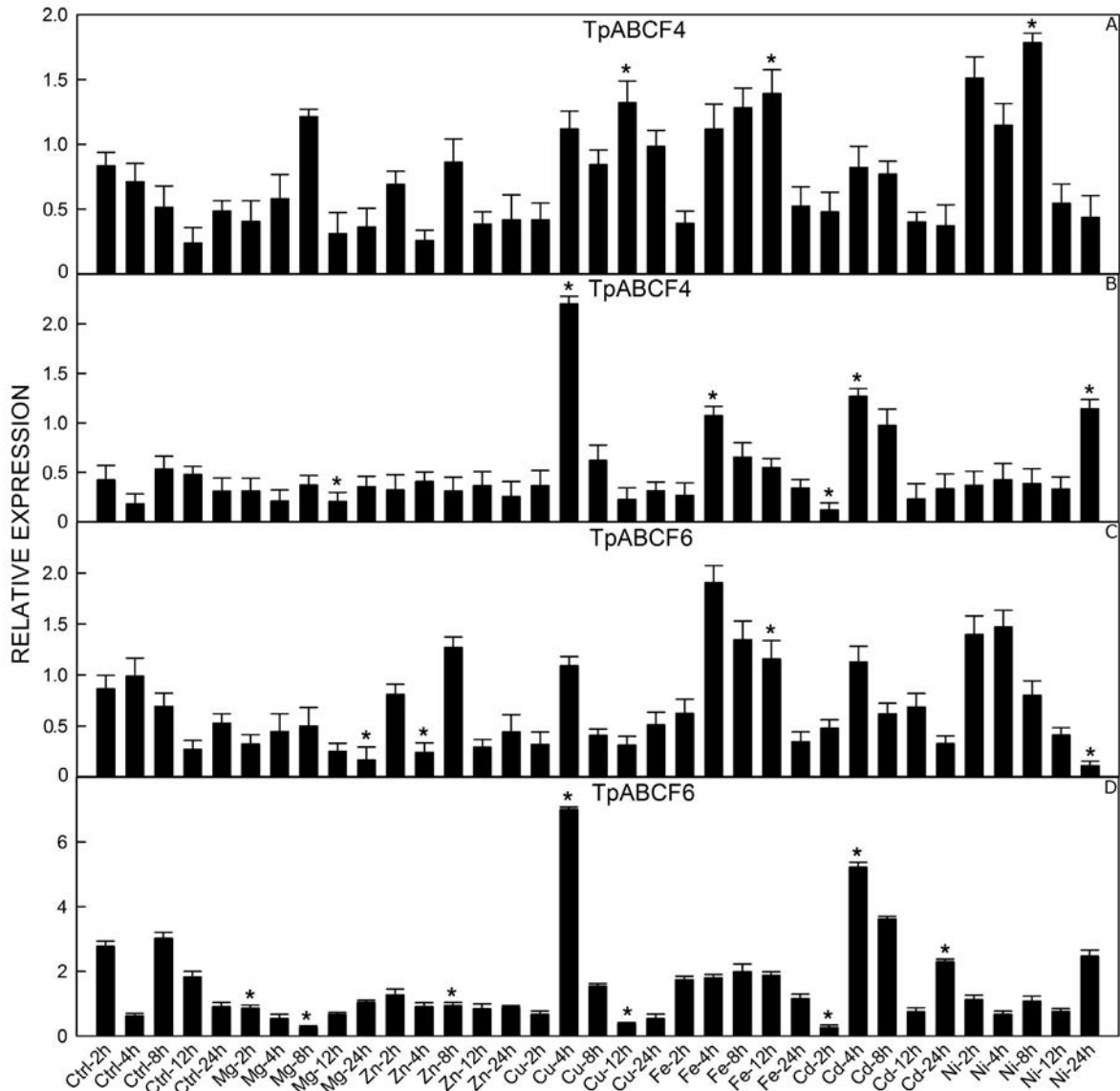

Fig. 7. Relative expressions of *TpABCF4* and *TpABCF6* in the roots (*A, C*) and leaves (*B, D*) under different metal stresses. Means ± SDs, *n* = 3, * - significant regulation > threefold or < 0.33-fold and *P* < 0.01.

GintABC1 of *Glomus intraradices*, *AtPDR8*, *Opp1* of *Staphylococcus aureus*, *AtABCC1*, *AtABCC2*, and *AtABC19*, are also regulated by other metals, which include Fe, Zn, Cu, Ni, Hg, Co, or Pb (Moons 2003, Kim *et al.* 2007, González-Guerrero *et al.* 2010, Park *et al.* 2012, Remy *et al.* 2013, Mentewab *et al.* 2014). Similarly to these results, the expressions of these 13 *ABC* genes were also differently regulated by Cu, Mg, Zn, Fe, and Ni. Meanwhile, most of the genes were regulated by a metal for at least two sampling times. Moreover, the

expressions of several genes, including *TpABCA3* under Cu stress in the leaves, *TpABCB13* under Fe stress in the roots, *TpABCB26* under Zn, Fe, and Cu stresses, *TpABCC1* under Fe stress, *TpABCD2* under Mg and Zn stresses, *TpABCE2* under Mg and Ni stresses, *TpABCG8* under Cu and Fe stresses, and *TpABCG42* under Mg, Fe, and Cu stresses, were regulated for at least three sampling times. These results suggest that the expressions of ABC transporters play important roles in metal transport and detoxification (Bovet *et al.* 2005, Park *et al.* 2012).
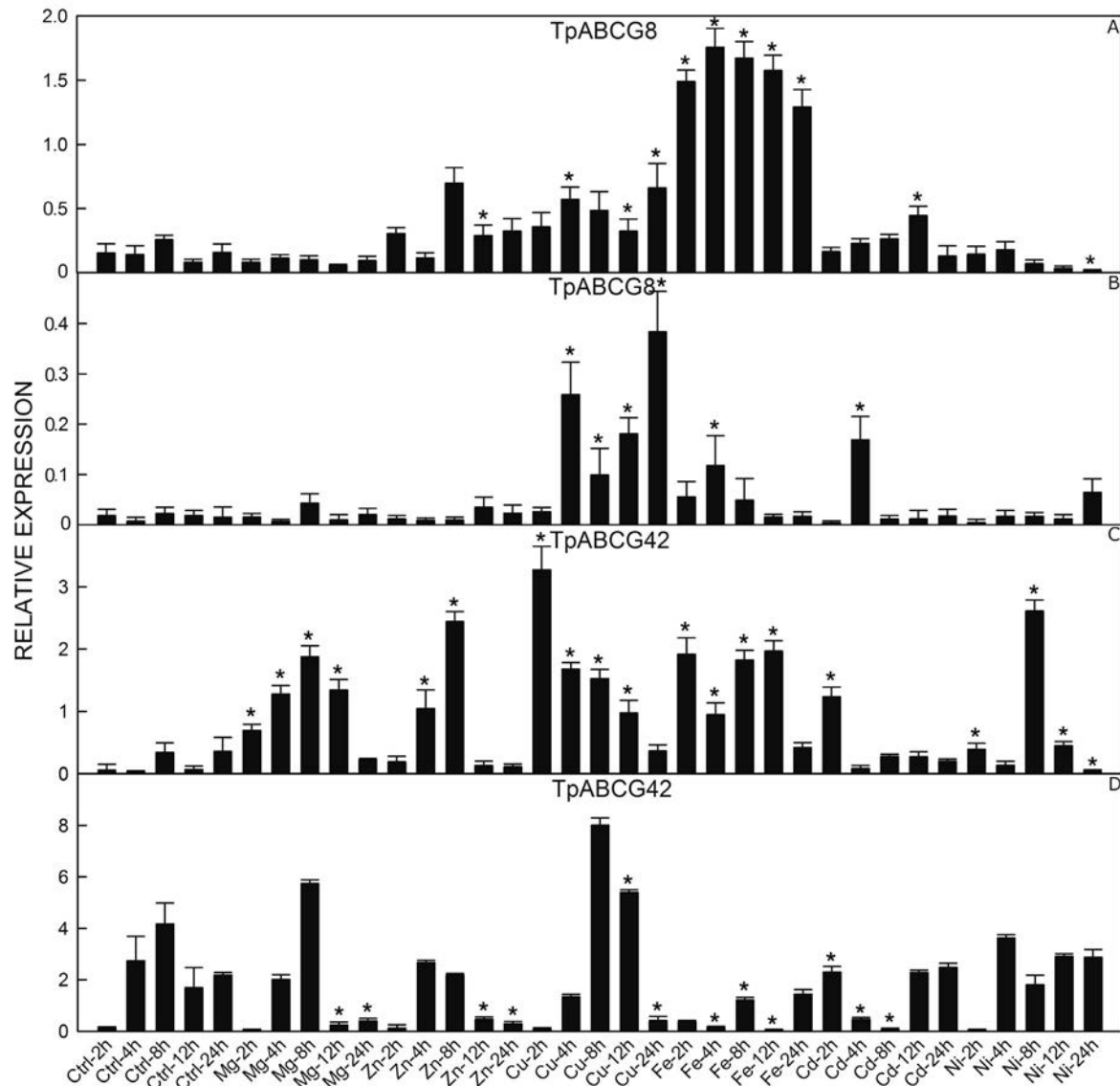
Fig. 8. Relative expressions of *TpABCG8* and *TpABCG42* in the roots (*A, C*) and leaves (*B, D*) under different metal stresses. Means ± SDs, $n = 3$, * - significant regulation > threefold or < 0.33-fold and $P < 0.01$.

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