DOI: 10.32615/bp.2020.125 BIOLOGIA PLANTARUM 64: 845-855, 2020

Genome-wide identification and expression analysis of the potato *ZIP* gene family under Zn-deficiency

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Abstract

Zinc deficiency is a worldwide problem for crops including potato (*Solanum tuberosum* L.), the fourth most important crop worldwide. The zinc/iron-regulated transporter-like protein (ZIP) transporter family is thought to play key roles in Zn uptake and transport. However, little is known about the potato ZIP family. In this study, 12 genes encoding members of the ZIP family were identified in the potato genome. The 12 *StZIP* genes were predicted to encode proteins of 220 - 407 amino acids harboring 5 - 9 putative transmembrane domains (TMDs), and 11 of these proteins had a variable region rich in histidine residues between TMDIII and TMDIV. A phylogenetic analysis divided the *StZIPs* into four groups on the basis of gene structure and conserved motifs. Furthermore, the *StZIP* expression profiles were determined under Zn-deficiency in both high and low Zn-content genotypes. Four differentially expressed genes, *StZIP6*, *-9*, *-11*, and *-12*, were identified in tubers of the two genotypes under Zn-deficiency, and *StZIP11* and *StZIP12* may have a more prominent function in Zn uptake and accumulation in potato tubers owing to their higher expressions. Thus, the results provide useful information for further studying the functions of *StZIP* genes.

Additional key words: *Solanum tuberosum*, transmembrane domains, Zn uptake and transport.

Introduction

Zinc is a key element in a large number of transcriptional regulatory proteins. It serves as a cofactor for many metalloenzymes and plays important roles in plant growth regulation, enzyme activation, gene expression and regulation, protein synthesis, photosynthesis, and saccharide metabolism (Marschner 1995, Guerinot 2000, Figueiredo *et al*. 2012). However, Zn deficiency is a widespread problem among crops (Hambidge 2000, Cakmak 2008), and approximately 40 % of the total land area of China is Zn deficient (Yang *et al*. 2007). Zn deficiency impairs Zn-dependent physiological functions, leading to abnormality in plant growth (Marschner 1995, Rengel and Graham 1995, Lombnaes and Singh 2003) and causing decreases in yield and nutritional quality (Marschner 1995, Yusuf *et al*. 2002, Sadeghzadeh 2013).

In plants, Zn, as a divalent cation or in complex with organic ligands, is translocated to the root xylem through symplast and apoplast pathways, and transported further through both xylem and phloem tissues (Haslett *et al*. 2001, Kabata-Pendias and Pendias 2001, Broadley *et al*. 2007). In recent years, several types of transporters, including P-type ATPase, multi-drug and toxic compound extrusion transporters, oligopeptide transporters, and zinc/iron-regulated transporter-like protein (ZIP) transporters, have been found to be involved in Zn uptake and translocation (Lochlainn *et al*. 2011, Hu *et al*. 2012, Pineau *et al*. 2012). The ZIP transporter family plays key roles in Zn uptake and translocation (Eide *et al*. 1996, Grotz *et al*. 1998, Pence *et al*. 2000, Wintz *et al*. 2003, Lin *et al*. 2009, Milner *et al*. 2013). Most ZIP proteins are predicted to have eight potential transmembrane domains (TMDs), and the amino and carboxy terminal ends of the protein are located on the outside surface of the plasma membrane (Guerinot 2000). There is a variable region between TMDs III and IV that is

Submitted 13 May 2020, *last revision* 6 August 2020, *accepted* 3 September 2020.
Abbreviations: *GSDS - gene structure display server tool*; *PGSC - Potato Genome Sequencing Consortium*; RH - *Solanum tuberosum* group *Tuberosum* RH89-039-16; DM - *Solanum tuberosum* group *Phureja* DM1-3 516 R44; TMD - transmembrane domain; ZIP - zinc/iron-regulated transporter-like protein.
Acknowledgements: The authors thank Lesley Benyon, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript. This work was supported by the National Natural Science Foundation of China's Youth Fund (31701486), Key-Area Research and Development Program of Guangdong Province (2020B020219002), Guangdong Provincial Science and Technology Program (2017B020232002), and The National Key Research and Development Program of China (2018YFD020080902). The first two authors contributed equally to this work.
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predicted to reside in the cytoplasm; this region contains a potential metal-binding domain, rich in histidine residues, which is associated with transport (Guerinot 2000, Mäser *et al*. 2001). To date, ZIP family members have been identified in bacteria, archaea, and all types of eukaryotes such as animals, plants, protists, and fungi (Mäser *et al*. 2001). In *Arabidopsis thaliana*, 15 ZIP members have been found, including *AtIRT1-3* and *AtZIP1-12*, which share sequence similarities of 38 - 85 % (Mäser *et al*. 2001). A phylogenetic analysis using amino acid sequence divided these ZIP proteins into four groups, with one of the groups clearly being more distantly related (Mäser *et al*. 2001). Among them, *AtIRT1-3*, *AtZIP1-3*, *-7*, *-11,* and *-12* were able to functionally complement a yeast Zn uptake-deficient mutant (Eide *et al*. 1996, Grotz *et al*. 1998, Guerinot 2000, Haslett *et al*. 2001, Vert *et al*. 2001, 2002, 2009, Lin *et al*. 2009, Milner *et al*. 2013). The expressions of some *ZIPs*, including *AtZIP1, -5*, *-9*, *-12,* and *AtIRT3*, increase under Zn-limiting conditions (Krämer *et al*. 2007). The overexpression of *AtZIP1* increases short-term Zn uptake and seed Zn content in barley and cassava (Ramesh 2004, Gaitánsolís *et al*. 2015). In rice, 16 *OsZIPs* members have been identified by homology cloning or a real-time PCR-based strategy (Ramesh and Schachtman 2003, Ishimaru *et al*. 2005, Yang *et al*. 2009, Chandel *et al*. 2010). Under Zn deficiency, the expressions of *OsZIP1*, *-3,-6,* and *-8*, which complement the phenotype of a Zn uptake-deficient yeast mutant, are upregulated in both roots and shoots (Yang *et al*. 2009, Lee *et al*. 2010a,b, Kavitha *et al*. 2015). Additionally, the expressions of *OsZIP2* and *OsZIP7* are specifically upregulated in roots and shoots, respectively (Ramesh and Schachtman 2003, Ishimaru *et al*. 2005). *OsZIP3,* located in the node, is responsible for unloading Zn from the xylem of enlarged vascular bundles (Sasaki *et al*. 2015). The ectopic expression of *OsZIP1* or *OsZIP4* increases the Zn content in tobacco and finger millet seeds or sweet potato tuber, respectively (Ramegowda *et al*. 2013, Shin *et al*. 2016). The overexpression of *OsZIP4* or *OsZIP5* results in a disarranged Zn distribution in rice (Ishimaru *et al*. 2007, Lee *et al*. 2010a). Thus, ZIP proteins play key roles in plant responses to Zn deficiency. Over the past decade, the functions of ZIP members have been identified and investigated in many species, such as *Glycine max* (Moreau *et al*. 2002), *Medicago sativa* (López-Millán *et al*. 2004, Stephens *et al*. 2011), *Hordeum vulgare* (Tiong *et al*. 2014), *Zea mays* (Li *et al*. 2013), and *Vitis vinifera* (Gainza-Cortés *et al*. 2012). However, ZIPs have not been explored in potato, even though it is a globally important crop (White *et al*. 2017). To provide key information for the further identification and functional analysis of *StZIP* genes in potato, 12 members of the ZIP family were identified from the potato genome, and their phylogenetic relationships, transmembrane domains, conserved protein motifs, gene structures, chromosomal locations, and promoters were analyzed. In addition, the tuber Zn content of 76 potato genotypes and a cluster analysis were performed. The differences in the *StZIP* expression patterns in high and low Zn-content genotypes in response to Zn deficiency were investigated.

Materials and methods

Identification of potato ZIP family members: Potato protein sequence data (DM_v3.4_pep_nonredundant) were downloaded from the *Potato Genome Sequencing Consortium* (*PGSC*, http://potato.plantbiology.msu.edu/integrated_-searches.shtml). The hidden Markov model (*HMM*) was used to identify potato ZIP candidates, and the hidden Markov model profile of ZIP (PF02535) was downloaded from *Pfam* (http://pfam.xfam.org). A *HMMER* search was carried out using *HMMER* software with e-value $\leq 1e^{-1e}$. The outputs of putative protein sequences were analyzed using *CDD* (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi), *Pfam*, and *SMART* (http://smart.embl-heidelberg.de/) to confirm the presence of conserved domains. All nonredundant and high-confidence genes were assigned as potato *StZIPs*. The gene locus, amino acid length, chromosomal location, and other information on each *StZIP* was acquired from *PGSC*. The exon/intron organization of *StZIP* genes were analyzed using the *Gene Structure Display Server* tool (*GSDS*, http://gsds.cbi.pku.edu.cn/). The TMDs of the ZIPs were predicted using *CCTOP* (http://cctop.enzim.ttk.mta.hu/?ref=labworm). Molecular mass and isoelectric point were calculated using the online program provided by the *ExPASy* website (http://web.expasy.org/protparam/).

Multiple sequence alignment and phylogenetic analysis: Multiple sequence alignment was performed using *Clustal X 2.0*. The phylogeny of the ZIP proteins was constructed using *MEGA v. 7.0* by the neighbor-joining method with 1 000 bootstrap replications. The phylogenetic tree was modified using the online software *EvolView* (https://evolgenius.info//evolview-v2/#login). For sequence logo analysis, the variable region sequences from potato, tomato, pepper, *Arabidopsis*, and rice were aligned, and the multiple alignment result was submitted to an online tool, *WEBLOG* (http://weblogo.berkeley.edu/logo.cgi).

Gene structure and conserved motif characterization of potato ZIPs: Conserved motif residues in potato ZIP sequences were determined using *MEME* suite with maximum motif number of 5, and minimum and maximum motif widths of 6 and 50, respectively (http://meme-suite.org/tools/meme). The predicated motif sequences were annotated using *CDD*, *SMART*, and *InterPro* (http://www.ebi.ac.uk/interpro/). The exon-intron structures of the *StZIP* genes were identified using *GSDS* (http://gsds.cbi.pku.edu.cn/).

Chromosomal location: The chromosomal positions of *StZIP* genes were obtained using the potato genome browser at *PGSC*. The approximate locations of *ZIP* genes were mapped to potato chromosomes using *Mapchart* software. Gene duplication was confirmed on the basis of two criteria: *1*) the length of the shorter aligned sequence covering >70 % of the longer sequence, and *2*) the similarity between the two aligned sequences being >70 % (Gu *et al*. 2002, Yang *et al*. 2008). Two genes separated

by five or fewer genes in a 100 kb chromosomal fragment were considered to be tandemly duplicated genes (Deng *et al*. 2014).

Promoter analysis: The 1 500 bp upstream sequences from the transcriptional start sites of *StZIP* genes were analyzed using *PlantCARE* (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/), and the results were modified using *GSDS*.

Transcriptional expression pattern analysis of *StZIP* genes: The transcriptional expression patterns of *StZIP* genes in different tissues were determined using RNA-seq data generated from *PGSC*. The read per kilobase of exon model per million reads mapped values of the *ZIP* genes were downloaded and $\log_2$-transformed, and the expression patterns were displayed using a heatmap generated by *HemI 1.0* (Deng *et al*. 2014).

Plant materials and treatments: Healthy tubers of potato (*Solanum tuberosum* L.) genotypes L14148-5 and Minshu1 were cut into uniform size samples and sterilized with 3 % (m/v) carbendazim. They were then planted in pots with moistened silica sand (one tuber per pot) and cultured in growth chambers at day/night temperatures of 25/20 °C, a 16-h photoperiod, an irradiance of 60 μmol m^{-2} s^{-1}, and a relative humidity of 50 %. After the potato seedlings emerged, the mother tubers were removed. Seedlings were divided into two groups. The first group was watered with Hoagland's solution as described by Fong and Ulrich (1969) once a week, and the second group was watered with the Hoagland's solution minus Zn once a week, representing Zn-deficiency condition. The leaves and tubers were sampled at 60 and 90 d after treatment, respectively, from three individual plants growing under Zn-sufficient and Zn-deficient conditions. All the samples were immediately frozen in liquid nitrogen and stored at -80 °C for further processing.

Determination of Zn content: Potato tubers were dried at 65 °C for 1 week. The 25-mg samples were wet-ashed with 5 cm^3 of HNO_3 and 1 cm^3 of $HClO_4$ at 150 °C for 5 h. The Zn content was measured using a flame atomic absorption spectrophotometer (*ZA3300*, *Hitachi*, Tokyo, Japan) at a wavelength of 213.86 nm.

Real-time PCR analysis was performed on a *CFX96* real-time PCR detection system (*Bio-Rad*, Hercules, USA) using *SYBR® Premix Ex TaqTM II* (*Tli RHaseH Plus*) (*TaKaRa*, Tokyo, Japan). All the reactions were carried out in a 25 mm^3 volume containing 1 mm^3 of cDNA as the template. The two-step thermal cycling conditions consisted of 95 °C for 30 s, followed by 39 cycles of 95 °C for 5 s, and 60 °C for 30 s. The *ef1-α* was used as an internal control. Each PCR assay included three biological replicates and three technical replicates. PCR data were evaluated using the $2^{-\Delta Ct}$ method. The specific primers used for each gene are listed in Table 2 Suppl.

Results

In total, 26 putative sequences were obtained by the HMMER search, and 12 sequences were identified as potato ZIP proteins after confirming the presence of characteristic conserved domains using CDD, Pfam, and SMART. The StZIPs were mainly characterized as having 2 - 4 exons encoding 220 - 407 aa, with molecular masses ranging from 23.67 to 43.41 kDa and isoelectric points ranging from 5.4 to 9.03. Detailed information on their characteristics is listed in Table 1. The *ZIP* genes were named *StZIP1* to *StZIP12* in accordance with their chromosomal locations.

The StZIP proteins were predicted to have five to 9 putative TMDs, and five StZIPs, StZIP5, -6, -8, -9, and -12, were predicted to have eight putative TMDs (Table 1, Fig. 1 Suppl.). All the StZIP proteins, except for StZIP11, had a long loop region between TMDIII and TMDIV that is referred to as the variable region. The variable region contained a potential metal-binding domain that is rich

Table 1. Features of *Solanum tuberosum zinc/iron-regulated transporter-like protein* (*StZIP*) genes identified in potato. TMD - transmembrane domain, pI - isoelectric point, Mr - molecular mass.

Gene name	Genomic ID	AA [aa]	No. of exons	No. of TMDs	pI	Mr [kDa]	Chromosome location
StZIP1	PGSC0003DMG400014936	348	3	9	6.50	37.239	chr02: 15695173,15697155
StZIP2	PGSC0003DMG400010343	281	2	6	8.81	30.673	chr02: 28265610,28267700
StZIP3	PGSC0003DMG400010344	331	3	9	8.48	35.691	chr02: 28278147,28279390
StZIP4	PGSC0003DMG400021155	360	3	9	9.03	38.874	chr02: 28347797,28350229
StZIP5	PGSC0003DMG400017732	354	3	8	8.16	38.436	chr02: 37391695,37394478
StZIP6	PGSC0003DMG400027159	320	2	8	5.96	35.244	chr05:48633733,48635684
StZIP7	PGSC0003DMG400002151	334	3	9	5.63	36.483	chr06: 4560296, 4563027
StZIP8	PGSC0003DMG400013109	340	3	8	6.14	36.434	chr07: 41345919, 41348870
StZIP9	PGSC0003DMG400013108	333	3	8	6.05	35.589	chr07: 41354134,41357585
StZIP10	PGSC0003DMG400011747	220	3	5	7.07	23.667	chr07: 41452935,41456033
StZIP11	PGSC0003DMG400022179	349	3	9	5.40	37.528	chr07: 55406651,55409430
StZIP12	PGSC0003DMG400029937	407	4	8	6.14	43.405	chr08: 35990888,35994505

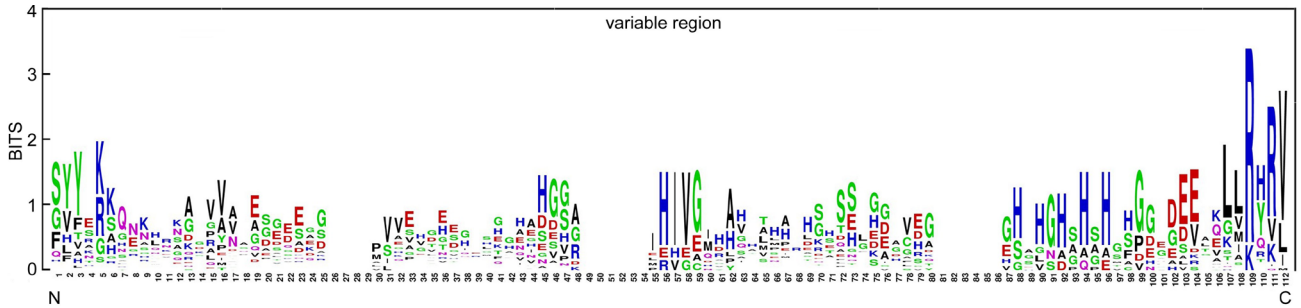

Fig. 1. Sequence logos showing variable regions of 12 zinc/iron-regulated transporter-like proteins (ZIPs) from potato, 10 ZIPs from tomato, 4 ZIPs from pepper, 15 ZIPs from *Arabidopsis,* and 10 ZIPs from rice.

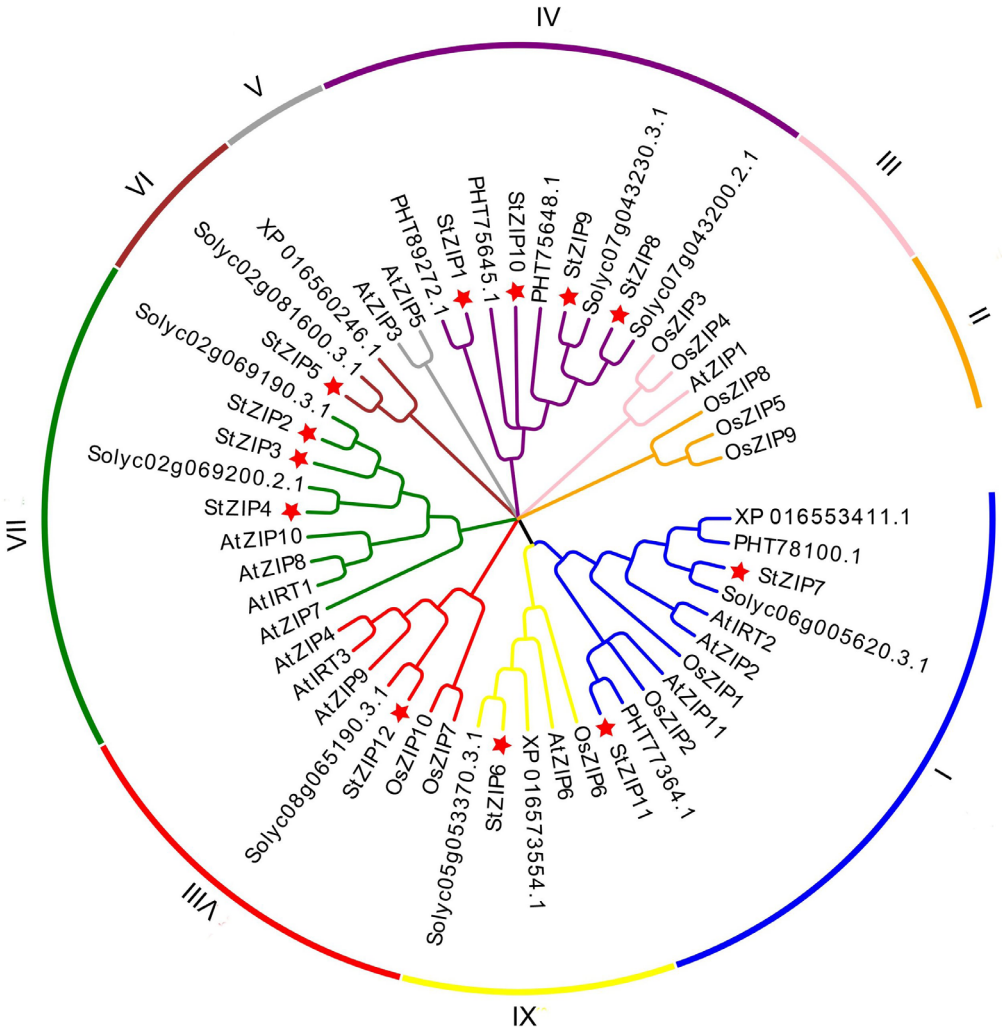

Fig. 2. A phylogenetic tree of zinc/iron-regulated transporter-like proteins (ZIPs) from *Arabidopsis*, rice, tomato, pepper, and potato constructed using the *MEGA7.0* software by the neighbor-joining method with 1 000 bootstrap replications. The nine subfamilies are distinguished by different colors.

in histidine. For example, in StZIP12, this sequence is HAHAAHHRHSHSHEH, and in StZIP8, this sequence is IHTHSHAHAH, suggesting a role in metal transport or regulation (Fig. 1, Fig. 1 Suppl.).

An unrooted phylogenetic tree was constructed using full-length amino acid sequences, with 10 sequences from rice, 14 sequences from *Arabidopsis*, 11 sequences from tomato, 5 sequences from pepper, and 12 sequences from potato. In total, 52 sequences were classed into 9 subgroups. The 12 potato ZIPs had members in 6 subgroups, except subgroups II, III, and V. Within the same subgroups, potato ZIP proteins were more closely

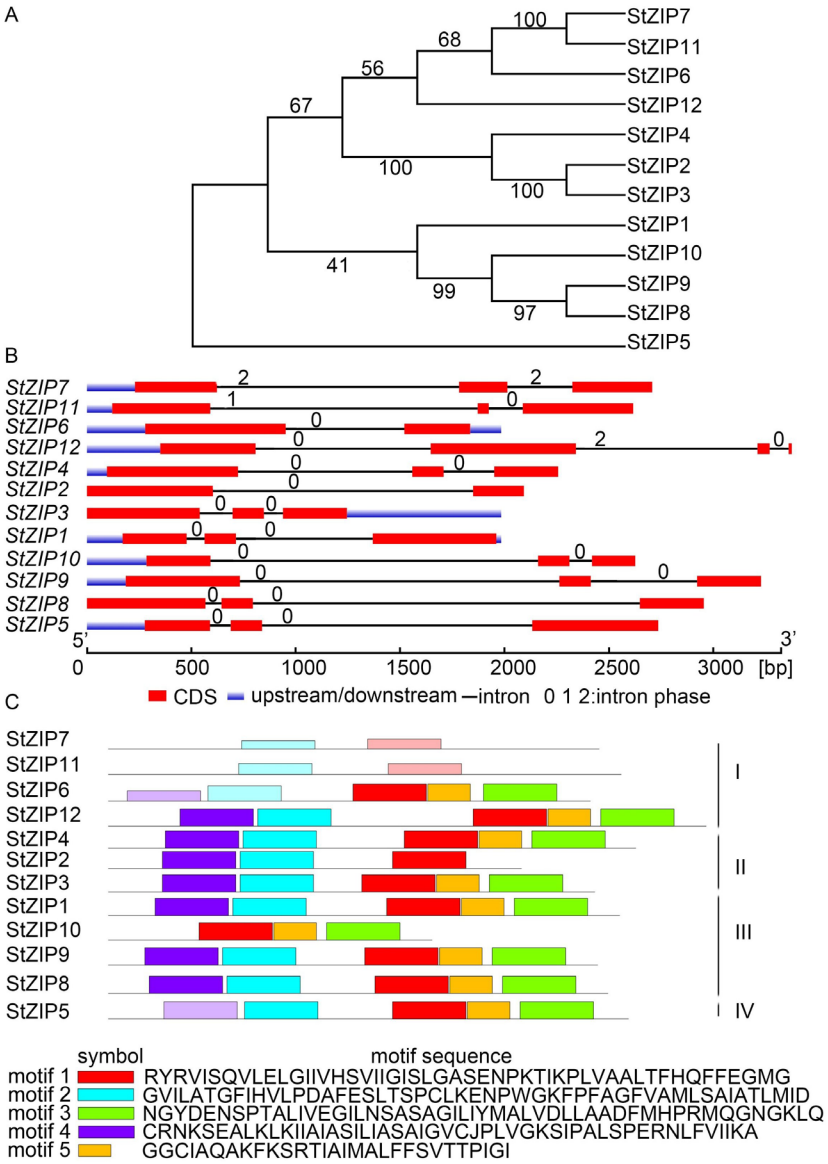

Fig. 3. The evolutionary relationships, gene structures, and functional motifs of the *Solanum tuberosum zinc/iron-regulated transporter-like proteins* (*StZIPs*). *A* - a phylogenetic tree constructed using the *MEGA7.0* software by the neighbor-joining method with 1 000 bootstrap replications. *B* - exon/intron distributions of corresponding *StZIP* genes detected by comparing the predicted coding sequences with their corresponding genomic sequences using *gene structure display server tool* online. *C* - motif composition of each StZIP protein. Motifs 1 - 5 are displayed as *differently colored boxes* with the corresponding sequence information for each motif. The lighter colors represent the lower similarity with the motif sequences.

related to tomato or pepper ZIP proteins than rice and *Arabidopsis* ZIP proteins. Subgroup I was the largest, with 11 members, while subgroup V was the smallest, containing only 2 members. Most ZIP proteins in the same subgroup shared similar exon-intron structures. For instance, subgroup IX had only one intron, subgroups VI and V had two introns, and subgroup VIII had three introns except for AtZIP9 (Table 1 Suppl.). In addition, *StZIP2*, *-3*, and *-4* located on chromosome 2, and *StZIP8-10* located on chromosome 7 clustered in subgroups VII and IV, respectively, suggesting that they might represent tandemly duplicated genes (Fig. 2).

Structures and phases of introns/exons were determined by the alignment of genomic DNA with the full-length cDNA of the *StZIPs* (Fig. 3*B*). The number of introns varied from one to three. Nine *StZIP* members (75 %) had two introns, two members (16.7 %) had only one intron, while only one member had three introns. Three intron phases, termed zero, one, and two, were generally present when splicing occurred after the third, first, and second nucleotides, respectively. Seven members, *StZIP1*, *StZIP3-StZIP5*, and *StZIP8-StZIP10,* had intron phase (0, 0), *StZIP7* had intron phase (2, 2), and *StZIP11* had intron phase (1, 0). *StZIP2* and *StZIP6* had only one intron with

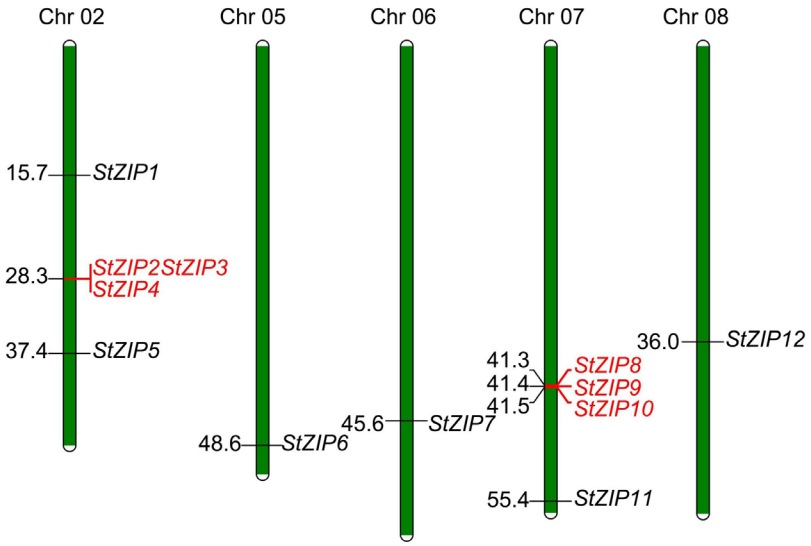

Fig. 4. Chromosomal locations of *Solanum tuberosum zinc/iron-regulated transporter-like protein* (*StZIP*) genes mapped using information available from the potato genome database. Tandemly duplicated genes are marked in *red,* the *scale* is in megabases.

intron phase (0) and *StZIP12* had the most introns with intron phase (0, 2, 0) (Fig. 3*B*).

The conserved motifs of the StZIPs were analyzed using *MEME* software. In total, five conserved motifs, having lengths of 29 to 50 aa, and one copy of all motifs was found in each StZIP protein. The motif sequences were annotated according to *CDD*, *SMART*, and *Pfam*, and motifs 1, 3, and 4 were predicted to form a transmembrane helical region. Each member contained different numbers of conserved motifs, ranging from two to five. Motif 1 was found in all StZIP proteins, and five conserved motifs were found in the majority of proteins (66.7 %). Three motifs were found in other members, such as StZIP10 and StZIP2, while only two motifs were found in StZIP7 and StZIP11. The different conserved motif profiles may indicate functional diversity. In addition, the StZIPs were subdivided into four groups on the basis of the phylogenetic analysis, and proteins in the same group of the phylogenetic tree shared similar motif structures (Fig. 3*C*).

The *StZIP* genes were unevenly distributed on five potato chromosomes. Chromosome 2 had most *StZIP* genes - five, chromosome 7 contained four members, while only one gene was found on the other chromosomes. The majority of *StZIP* genes were located on the proximate or distal ends of the chromosomes. On the basis of the two criteria, six (50 %) *StZIP* genes were confirmed to be tandemly duplicated genes. *StZIP2*, *-3*, and *-4* formed a duplicated gene cluster, and *StZIP8*, *-9*, and *-10* formed another duplicated gene cluster (Fig. 4).

To understand the transcriptional regulatory mechanisms of the *StZIP* gene family, the promoter region's *cis*-regulatory elements located in the sequence 1 500-bp upstream of the transcriptional start sites were analyzed using *PlantCARE*. Many *cis*-regulatory elements were identified in this region. Most *cis*-regulatory elements were associated with important physiological processes such as in defense and responses to stress, radiation, and hormones. For example, the ATCT motif, Box4, GATA motif, and GT1 motif are involved in radiation responsiveness; myoblastosis and TC-rich motifs are involved in defense and stress responsiveness; ABRE is involved in ABA responsiveness; and the TGA element is involved in auxin responsiveness. In addition, other uncommonly distributed *cis*-regulatory elements that are functionally associated with transcription modulation, endosperm and meristem expression, leaf differentiation and development, and circadian control were found. Thus, *StZIP* genes may be regulated by various environmental and developmental changes. Duplicated gene pairs did not share similar *cis*-element distributions. These observations suggest the involvement of duplication events in conferring neofunctionalization to *StZIP* genes during their divergence and evolvement (Fig. 5).

To analyze the expression patterns of *StZIP* genes in various tissue types and organs, the read per kilobase of exon model per million mapped reads values of two potato species, *Solanum tuberosum* group *Tuberosum* RH89-039-16 (RH) and doubled monoploid *Solanum tuberosum* group *Phureja* DM1-3 516 R44 (DM), were downloaded from *PGSC* and a heatmap was generated. *StZIP5*, *-9*, *-11*, and-*12* had relatively high expression in all tissue types for both RH and DM. *StZIP1* was highly expressed in flowers, leaves, and stem, but not tubers, in both RH and DM. The expressions of *StZIP2, -3,* and *-4* were mainly detected in flowers, suggesting that they may be related to flower development. In addition, *StZIP2* showed a relatively high expression in the root of DM. *StZIP6* showed a moderate expression in the stem of RH and in the flower and roots of DM. *StZIP7* was highly expressed in the root and stolons of DM. *StZIP10* exhibited moderate expression in RH leaves and stem. *StZIP8* expression was nearly undetectable in all the tissue types except for RH leaves. Thus, *StZIP* members exhibited very different expression profiles in different organs for the two potato species. *StZIP1*,*-4*,*-5*,*-9*,*-10*,*-11*, and *-12* have similar expression patterns in these two potato genotypes, while *StZIP2*,*-3*,*-6*,*-7* and *-8* showed

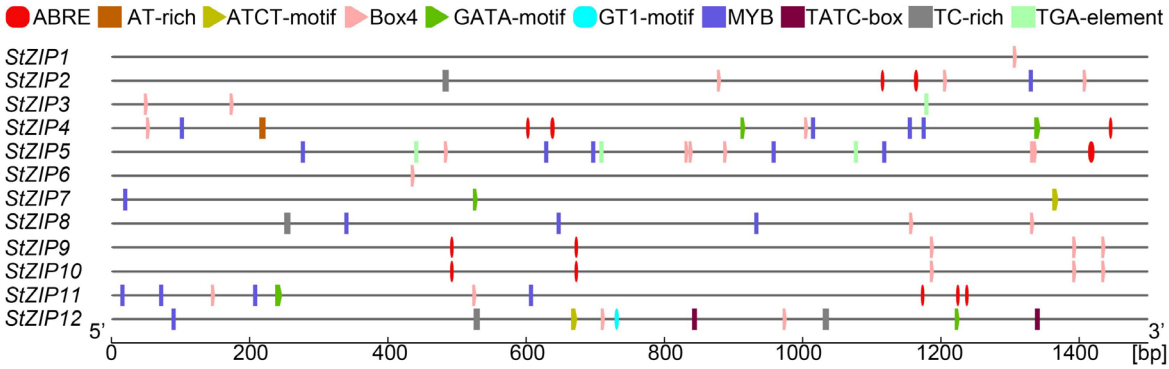

Fig. 5. Promoter analysis of *Solanum tuberosum zinc/iron-regulated transporter-like protein* (*StZIP*) genes. The *legend* shows different stress-response elements located in the 1.5 kb 5' upstream region of each *StZIP* gene.

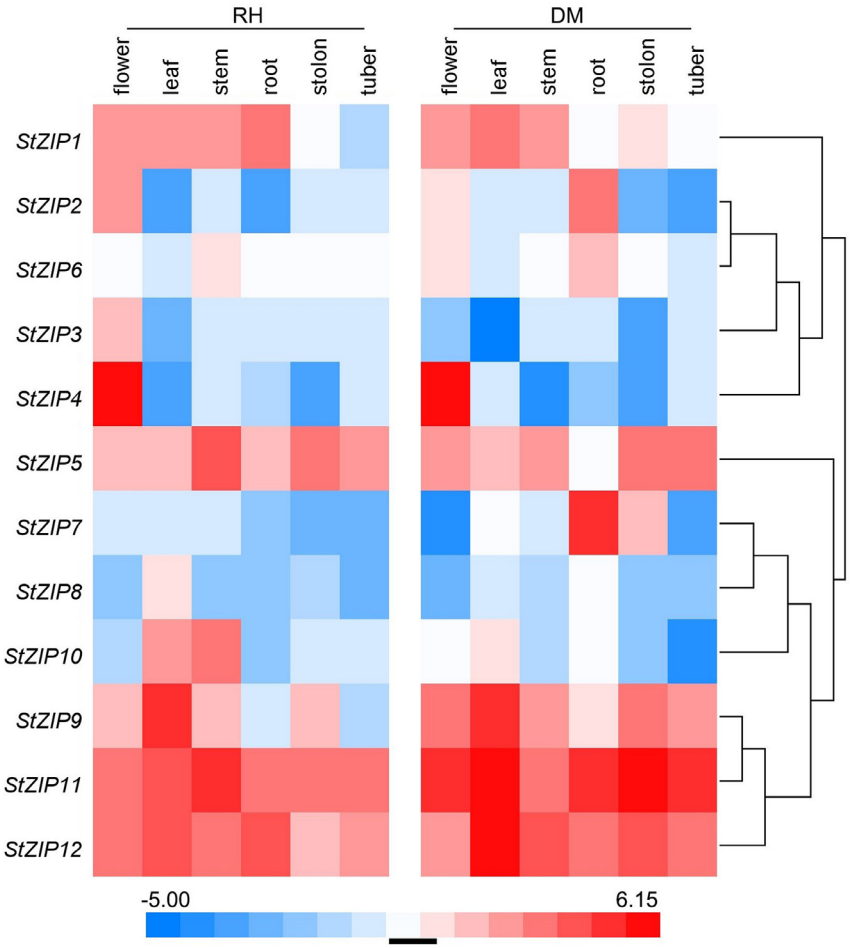

Fig. 6. Expression profiles of *Solanum tuberosum zinc/iron-regulated transporter-like protein* (*StZIP*) genes in different potato organs and tissue types. A heatmap generated by *HemI 1.0* using reads per kilobase of exon model per million reads mapped values of all the *ZIP* genes that were downloaded from the *Potato Genome Sequencing Consortium* and $\log_2$-transformed. DM - doubled monoploid *Solanum tuberosum* goup *Phureja* DM1-3, RH - *Solanum tuberosum* group *Tuberosum* RH89-039-16.

significantly different expressions (Fig. 6).

The expression patterns of *StZIPs* in potato were further studied to understand their roles in Zn uptake and translocating under Zn-deficiency conditions. First, the tuber Zn content of 76 potato genotypes were evaluated, and the genotypes clustered into four subgroups on the basis of their Zn content. Subgroup I, containing eight potato genotypes, had the highest Zn content ranging from 31.44 to 46.90 mg kg^{-1}(f.m.). Subgroup II, containing 13 genotypes, was a middle-to-high Zn content group with Zn content ranging from 25.07 to 29.9 mg kg^{-1}(f.m.). Subgroup III, containing 18 genotypes, was a middle

Table 2. Tuber Zn content in two potato genotypes under normal (CK) and Zn-deficiency conditions. ** - significant differences at $P \leq 0.01$.

	CK	Zn Deficiency
L14148-5	39.50 ± 1.64	37.62 ± 2.69**
Minshu1	17.00 ± 0.43	15.44 ± 0.45**

Zn content group with Zn content ranging from 20.48 to 23.76 mg^{-1}(f.m.), and subgroup VI, containing the most genotypes, was the lowest Zn content ranging from 12.69 to 20.08 mg kg^{-1}(f.m.) (Fig. 1 Suppl.).

Two genotypes, L14148-5 in subgroup I and Minshu1 in subgroup VI, were selected to explore the expression patterns of *StZIP* members. Additionally, we detected their tuber Zn content under Zn-deficiency. Even under these conditions, L14148-5 maintained a twofold greater content of Zn in tubers than Minshu1 (Table 2).

The expressions of three genes, *StZIP5*, *-11,* and *-12*, were higher in leaves and tubers compared with the other genes. In leaves, the expression of *StZIP1* was induced, while the expressions of *StZIP5*, *-7*, *-8*, *-10*, and *-11* were repressed under Zn-deficiency in both genotypes. The expressions of some other genes presented genotype-based differences, such as *StZIP3* and *StZIP9*, which were reduced in L14148-5 but induced in Minshu1 under Zn deficiency. *StZIP4* showed an opposite expression pattern in response to Zn deficiency, being induced in L14148-5 and repressed in Minshu1. In addition, some genes showed expression changes in only one genotype. For example, under Zn-deficiency, the expression of *StZIP2* was repressed only in L14148-5, the expression of *StZIP12* was induced only in Minshu1, and the expression of *StZIP6* was repressed only in Minshu1. With regard to tubers, the expressions of *StZIP1*, *-2*, *-3*, *-4*, *-7*, and *-8* were induced, while *StZIP5* and *StZIP10* were reduced under Zn-deficiency in both genotypes. The expressions of four other genes, *StZIP6*, *-9*, *-11*, and *-12*, showed genotypic differences in response to Zn deficiency, and were increased in L14148-5 but decreased in Minshu1 (Fig. 7).

Discussion

In this study, we identified 12 ZIP members in potato (Table 1). There are 16 identified ZIP members in *Oryza sativa*, 15 in *Arabidopsis thaliana*, and 12 in *Citrus reticulate*, which indicate that the ZIP family is relatively small. The protein lengths of the potato, *Arabidopsis,* and orange ZIP members are 220 - 407 aa, 326 - 425 aa, and 334 - 419 aa (Fu *et al*. 2017). Potato ZIP members were predicted to have 6 - 9 TMDs, with the majority having 8 TMDs (Table 1, Fig. 1 Suppl.). Between TMDIII and TMDIV in all the *StZIPs*, except *StZIP11* (Fig. 1 Suppl.), there is a variable region that is rich in histidine that thought to be a cytoplasmic metal ion-binding site (Guerinot 2000). The StZIP11 similar to AtZIP7, AtZIP11, MtZIP1, MtZIP7, and PtZIP2 (López-Millán *et al*. 2004, Fu *et al*. 2017), was found that the histidine-enriched region can be found at other different locations (Eng *et al*. 1998). StZIP12 has the greatest number of histidine residues in the variable region, which implies that it has a strong ability to bind

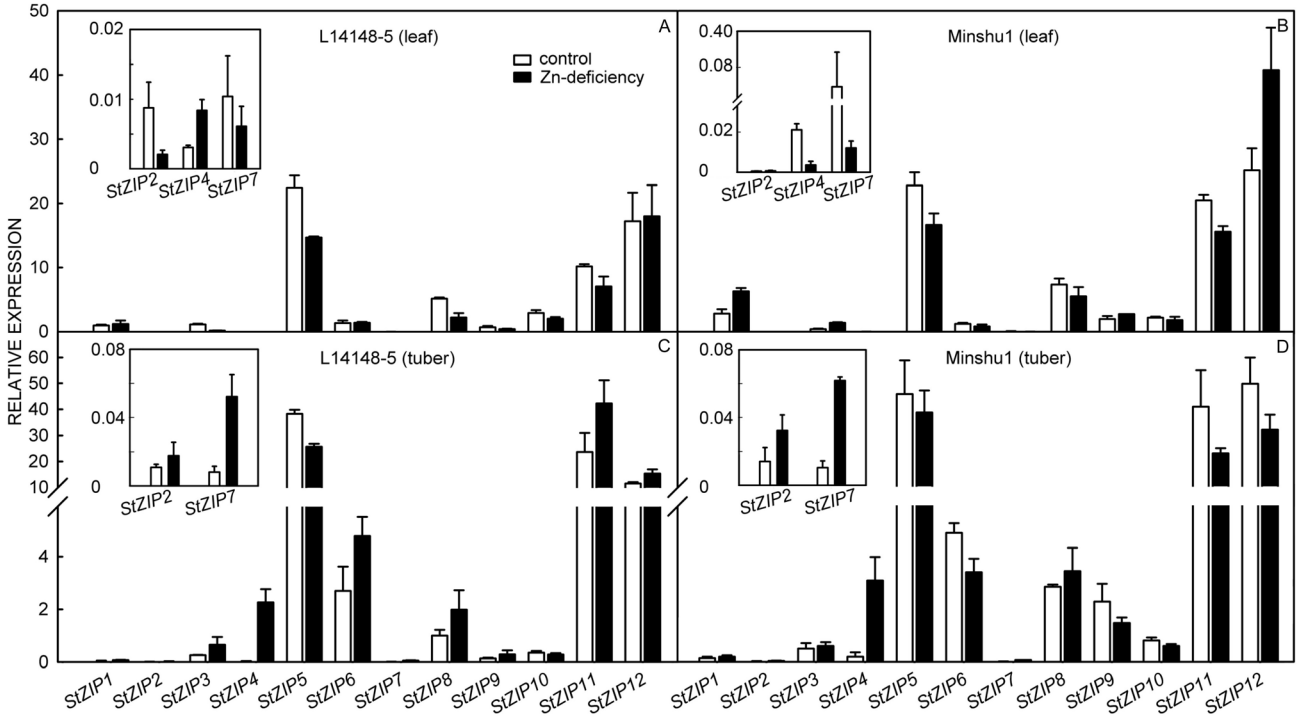

Fig. 7. Relative expressions of *Solanum tuberosum zinc/iron-regulated transporter-like protein* (*StZIP*) members in tubers and leaves of two potato genotypes in response to Zn deficiency. Values are means ± SEs, $n = 5$.

metal ions (Fig. 1). The 12 identified *StZIP* genes clustered into 4 subgroups, similar to the *ZIP* genes of *Arabidopsis* (Mäser *et al*. 2001). *StZIP2*, *-3*, and *-4* are duplicated genes that cluster into the same phylogenetic subgroup and share a similar gene structure (Figs. 2 - 4). They were found to be highly expressed in flower organs, suggesting that they function in flower development (Fig. 6). Real-time PCR results showed that the expressions of *StZIP2, -3, -4* were induced in tubers by the Zn deficiency, but their expressions in leaves showed genotype-based differences. *StZIP3* expression was decreased in L14148-5, but increased in Minshu1. *StZIP4* showed the opposite expression pattern to *StZIP3*, being induced in L14148-5 and repressed in Minshu1. The expression of *StZIP2* was repressed only in the high Zn content genotype L14148-5 (Fig. 7). There was another cluster of duplicated genes, *StZIP8*, *-9*, and *-10* (Fig. 4). *StZIP8* was weakly expressed in leaves and roots, and *StZIP9* had a relatively high expression in many tissues. The expression of *StZIP10* was observed in leaves, petioles, and stems (Fig. 6). In leaves, *StZIP8* and *StZIP10* were repressed under Zn-deficiency in both genotypes, While *StZIP9* was decreased in L14148-5, but increased in Minshu1. In the tubers of both genotypes under Zn-deficiency condition, *StZIP8* was induced, while *StZIP10* was repressed. *StZIP9* expression also showed genotype-based differences, being increased in L14148-5, but decreased in Minshu1, in response to the Zn-deficiency (Fig. 7). The functional diversity of the duplicated genes suggests that they may have evolved separate functions. Similar results were found in the research of duplicated genes in *Poncirus trifoliata* L. Three pairs of *PtZIPs* are both phylogenetically and physically close; however, there are no obvious similarities in their expression patterns and yeast complementation (Fu *et al*. 2017). In other plants, like *Arabidopsis* and rice, the expression of some ZIPs are induced by Zn deficiency. *AtZIP1*, *-5*, *-9*, and *-12,* as well as *AtIRT3*, increase under Zn-limiting conditions (Krämer *et al*. 2007), while OsZIP7 is only upregulated in shoots (Ramesh and Schachtman 2003, Ishimaru *et al*. 2005). *OsZIP1* - *OsZIP6* and *OsZIP8* are upregulated in roots and shoots under Zn-deficiency and complement a Zn uptake-deficient yeast mutant (Ramesh and Schachtman 2003, Ishimaru *et al*. 2005, Yang *et al*. 2009, Lee *et al*. 2010a,b, Kavitha *et al*. 2015). In our research, we also found that some *StZIPs* were induced under Zn-deficiency, with *StZIP1* induced in leaves and tubers, and *StZIP2*-*StZIP4*, *StZIP7*, and *StZIP8* induced in the tubers of both genotypes. We also found that the expression of some genes showed genotype-based differences in response to Zn-deficiency. *StZIP3* and *StZIP9* were only induced in the leaves of Minshu1*,* while *StZIP6* and *StZIP4* were only induced in the leaves of L1418-5. Additionally, *StZIP9*, *-11*, and *-12* were increased in tubers of L14148-5 but decreased in tubers of Minshu1 (Fig. 7). Compared with normal growth condition, four genes, *StZIP6*, *-9*, *-11*, and *-12,* were differentially expressed in potato tubers among high and low Zn content genotypes under Zn-deficiency (Fig. 7). The expressions of these four genes increased in L14148-4 and decreased in Minshu-1. In leaves, their expressions were unchanged or decreased under Zn deficiency compared with under normal growth condition in both genotypes (Fig. 7). The Zn content in the tubers of L14148-4 was more than twofold greater than in tubers of Minshu-1 under normal growth condition, and the former maintained the higher Zn content even under Zn-deficiency (Table 2). Thus, this indicates that the four differentially expressed genes, *StZIP6*, *-9*, *-11*, and *-12*, may have pivotal roles in Zn absorption and accumulation in potato tubers. Furthermore, *StZIP6* and *StZIP9* showed weak expression, while *StZIP11* and *StZIP12* showed much higher expressions in the leaves and tubers of both genotypes. The expression of *StZIP11* was more than fivefold greater than those of *StZIP6* and *StZIP9*, and the expression of *StZIP12* was more than five- and 12-fold greater than those of *StZIP6* and *StZIP9*, respectively (Fig. 7). *StZIP11* and *StZIP12* also had relatively high expressions in all the tissue types in RH and DM (Fig. 6), which suggests that *StZIP11* and *StZIP12* have prominent functions in the Zn uptake and accumulation in potato tubers.

Conclusions

Twelve ZIP genes were identified and clustered using the potato genome for the first time. We systematically identified ZIP family members from the potato genome, and analyzed their phylogenetic relationships, conserved protein motifs, and chromosomal locations, and we investigated the expression patterns of *StZIPs* in high and low Zn-content potato genotypes under the Zn deficiency. The results indicated that *StZIP* genes play important roles in Zn uptake and translocation. In future studies, we will further investigate the functions of StZIPs in response to Zn deficiency in potato in more detail.

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