INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0366/2020/24–6–1533–1542
DOI: 10.17957/IJAB/15.1592
http://www.fspublishers.org

Full Length Article

Genome-Wide Identification and Expression Analysis of the Zinc Transporter Protein ZIP Family in Potato (*Solanum tuberosum*)

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Received 09 March 2020; Accepted 09 June 2020; Published 10 October 2020

Abstract

The *ZIP* gene family is a metal transporter capable of transporting various cations, including iron, manganese and zinc. Metal transport and balance in plants are very important for growth and development. However, the comprehensive analysis of *ZIP* genes has not been reported in potato (*Solanum tuberosum* L.). Based on the whole potato genome data, the members of potato *ZIP* gene family were identified and systematically analyzed. In total, 29 ZIP proteins from *S. tuberosum* were identified. Through the establishment of phylogenetic tree, ZIP proteins were divided into three subgroups. Gene structure analysis showed that many genes had multiple introns. Motif analysis showed that these StZIP proteins had similar motif composition patterns. Twenty-nine *StZIP* genes were located on 7 chromosomes. A cis-element identified that many *StZIPs* also contain abiotic stress-related elements. A heatmap of the *StZIP* gene family identified that the genes, only *StZIP12*, *StZIP17* and *StZIP26* were expressed in all tissues and organs. A representative member *StZIP12* was selected for screening its expression characteristics to identify whether it was induced by *Ralstonia solanacearum* and abscisic acid (ABA) treatment. Quantitative real-time polymerase chain reaction (qRT-PCR) showed a positive result, which strongly suggested that the gene might be involved in potato response to bacterial wilt associated with ABA signaling pathway. Tissue localization showed that *StZIP12* was mainly expressed in phloem and leaf vascular bundles of stem vascular system. These results laid a foundation for further experimental cloning and functional verification, as well as the study of the molecular mechanism of potato resistance to bacterial wilt.

Keywords: *Solanum tuberosum*; ZIP; Bioinformatics analysis; ABA; *Ralstonia solanacearum*

Introduction

Zinc is not only an essential substance for regulating proliferation and metabolism, but also an intracellular second messenger and the cellular level of ionic, zinc homeostasis in cells are controlled by multiple transporters, including distinct families, such as Zn-regulated transporter, iron (Fe)-regulated transporter-like proteins (ZIPs), metal tolerance proteins (MTPs), and heavy metal ATPases (HMAs) (Palmer and Guerinot 2009; Thomas *et al.* 2018; Gao *et al.* 2019). In mammals, zinc homeostasis is primarily regulated by ZIP and ZnT zinc transporters, the importance and functions especially on human diseases of these transporters had been well reviewed (Thingholm *et al.* 2020) while there are relatively few studies on zinc transporter protein such as ZIP in plants. Recent studies have revealed the molecular mechanisms governing the uptake and accumulation of Zn in tobacco (Kozak *et al.* 2019), rice (Gao *et al.* 2019; Wang *et al.* 2020) and *Arabidopsis*, oxidative and biotic stress responses in the *Arabidopsis* (Scheepers *et al.* 2020). ZIP transporter is predicted to have 8 transmembrane domains. The metal binding domain provided by the histidine residue will form a very conservative histidine amphiphilic helix structure, which may be part of the transport of metal binding sites in the membrane. If there was a mutation in the residue, the transport function will disappear (Guerinot 2000).

Sixteen members of the ZIP gene family have been identified on the x`whole genome of the model plant *Arabidopsis thaliana* (Milner *et al.* 2013). ZIP has been shown to be related to the absorption and transport of metals by plants (Grotz *et al.* 1998; Plaza *et al.* 2007; Pedas *et al.* 2008; Assunção *et al.* 2010). *A. thaliana ZIP1*, *ZIP3* and *ZIP4* restored zinc uptake by yeast Zn-uptake mutants *Dzrt1* and *Dzrt2*, and were considered to play an important role in transport of zinc. When zinc is deficient, the expression of *ZIP1* and *ZIP3* increased in roots, which indicated that they played an important role in zinc transport while *ZIP4* was expressed in roots and buds at the same time, indicating that it transports zinc in cells (Grotz *et al.* 1998; Guerinot 2000).

To cite this paper: Yu R, Y Chang, Y Song, T Tian, H Wang, G Gao (2020). Genome-wide Identification and expression analysis of the zinc transporter protein zip family in potato (*Solanum tuberosum*). *Intl J Agric Biol* 24:1533–1542

A total of 17 ZIP family members were predicted in the whole genome of *Oryza sativa*, eight of these genes have been reported. The *ZIP* gene-encoded polypeptide in rice has approximately 276–687 amino acids and contains 8 transmembrane (TM) domains (Yang *et al.* 2009). Ninety-four per cent of the protein predictions had the typical secondary structure of the ZIP family. Most ZIP proteins are located on the cell membrane, maybe *OsZIP10* is located in the chloroplast, and *OsZIP16* is located in the vacuole. When rice was zinc deficient, *OsZIP1* and *OsZIP3* were up-regulated. These results suggest that they are zinc transporters regulated by zinc on the plasma membrane and are responsible for zinc transport (Ramesh *et al.* 2004).

Three new zinc transporters of *Hordeum vulgare*, *HvZIP3*, *HvZIP5* and *HvZIP*8, have 350–362 amino acids, and their encoded proteins are highly similar to ZIP family proteins. In barley roots, *HvZIP8* was expressed under normal conditions, while *HvZIP3* and *HvZIP5* were expressed in the absence of zinc. These results proved that *HvZIP3*, *HvZIP5* and *HvZIP8* were zinc transporters and were involved in the balance of zinc in barley roots (Pedas *et al.* 2009).

Thirty-three genes related to zinc and iron transport have been identified in corn (*Zea mays*) genome, but only 9 of them belong to the ZIP family. There are 7–9 transmembrane domains in the *ZmZIP* family. For example, *ZmZIP4* cDNA, with a full length of 1161 bp, is composed of a polypeptide with 386 amino acid residues, contained 7 transmememal domains, and its encoded protein is located on the lipid membrane (Xu *et al.* 2010).

Potato is one of the three food crops that human beings depend on for survival (Bali *et al.* 2018). It has rich nutrition, strong adaptability, good yield, rich nutrition, high economic benefits, and is suitable for processing (Bali *et al.* 2018). It plays an important role in the structure of food safety. With the wide application of bioinformatics approaches, many gene families in potato have been identified and completed related bioinformation analysis, such as SBP transcription factor family (Kavas *et al.* 2017) and ARF family (Song *et al.* 2019). However, the *ZIP* gene family has not been reported in potatoes, but only in *Arabidopsis*, rice, barley and corn. In this study, taking advantage of the DM potato reference genome, we conducted a genome-wide, comprehensive analysis of ZIP family genes in potato. A total of 29 *StZIP* (*Solanum tuberosum* ZIP) genes were identified, and the physical and chemical characteristics, genomic structures, chromosomal locations, evolutionary relationship, expression profiles of StZIP family were investigated in detail. In our previous work, a suppression subtractive hybridization (SSH) cDNA library library was created (Kong *et al.* 2016) to enrich the up-regulated genes induced by *R. solanacearum* in potato. A *StZIP12* gene was found, induced by biotic and abiotic stress, and analyzed in combination with the results of bioinformatics. This will lay a foundation for further research on the function of this gene.

Materials and Methods

Plant material and growth conditions

The potato (diploid genotype ED13) plants used in this study were provided by the Chinese Academy of Agricultural Sciences (CAAS). Potato ED13 genotype was resistant to bacterial wilt (Pang *et al.* 2019). Aseptic tuber ED13 was planted in pots containing 450 mL peat and vermiculite (3: 1, volume ratio) (10 cm, in diameter and 15 cm in height). The culture conditions were as follows: 16 h light (24°C)/8 h dark (18°C) photoperiod (light intensity approximately 3,000 lx) with 75% relative humidity (Pang *et al.* 2019).

Identification and characterization of potato *StZIP* family

Referring to the previous methods (Peng *et al.* 2019), the complete potato *StZIP gene* was obtained. All *StZIP* gene candidates were analyzed using the Hidden Markov Model (HMM) (Liang *et al.* 2016). Physical properties such as peptide length, isoelectric point and molecular weight, and subcellular localization were completed by ExPASy and PSORT online tools.

Phylogenetic tree analysis

A phylogenetic tree was constructed with MEGA 7 using Neighbor-Joining (NJ) method (Dereeper *et al.* 2010).

Conserved motif analysis, chromosome mapping and gene structure analysis of *StZIP*

The conserved domain of the protein was analyzed by online software MEME (Zou *et al.* 2019). The chromosomal locations information was retrieved from potato genome data and these data were downloaded from the phytozome (Liang *et al.* 2017). The gene structure of *StZIP* was analyzed by GSDS website.

Digital expression analysis of *StZIP* protein

Based on the digital expression data retrieved by previous methods (Nussbaumer *et al.* 2014), the expression of *StZIP* members in four tissues and developmental stages was studied.

Promoter sequence analysis

The base of the 5′ upstream 2000 bp of the initiation codon ATG of the *StZIP* gene family was obtained from the potato genomic DNA, and then Plant CARE was used to search the promoter region in the download sequence, to analyze each binding site and predict its potential cis-acting elements (Lescot *et al.* 2002).

Hormone treatment

According to the previous work (Blaudez *et al.* 2003), Five pots containing five potato plants at the seven or eight compound leaf-stage were sprayed with 100 μmol/L ABA solution respectively with distilled water as control. After spraying, all samples are covered with black plastic bags to maintain humidity and prevent hormone volatilization. The aboveground parts of plants were sampled at 0, 24, 48, 72, 96 and 120 h after treatment, frozen in liquid nitrogen and stored at -80 C for RNA preparation (Denancé *et al.* 2013).

Inoculation

Potato seedlings of 7–8 leaf age were treated with 10^8 cfu/mL (OD600=0.2) solution and inoculated with root injury irrigation method (He *et al.* 1983). Control plants were inoculated with an equal volume of water. Stem samples were collected at 6, 12, 24, 36, 48, 60, 72, 84, 96 and 108 h after the inoculation, wrapped with aluminum foil, placed in liquid nitrogen for 20 min.

Quantitative real-time polymerase chain reaction

In our previous work, a suppression subtractive hybridization (SSH) cDNA library was created and the EST of the *StZIP12* gene was found to up-regulated induced by *R. solanacearum* in potato. qRT-PCR reference Schmittgen'S method (Schmittgen and Livak 2008), potato Actin gene is internal reference gene (GenBank Accession: X55747).

The subsequent qRT-PCR was completed with the following primers: StZIP12-F: GTTGCCATCGGAATCATAATCG, StZIP12-R: ATGAGACTTGTCAATCG AGACC; internal reference; Actin-F: TATAACGAGCTTCGTGTTGCAC, Actin-R: ACTGGCATACAGCGAAAGAACA. The fragment size of PCR product is 168 bp. The qRT-PCR reaction procedure was as follows: preheating at 94°C 2 min, 94°C cycle 5 s, 58°C cycle 15 s, 72°C cycle 10 s, 45 cycles, and the experiment was repeated 3 times.

Fluorescence *in situ* hybridization

The bacterial liquid of *R. solanacearum* strain PO41 (Biovar 2, race 3, 10^8 colony forming units/mL) was used to infect ED13 seedlings. After 48 hours of inoculation, a small number of leaves and 2–3 stem segments (6–7 cm), were collected from each seedling and put into the 10 mL centrifuge tube. The plant material was immersed in a fixative comprising 85 mL 50% ethanol, 10 mL 37% formaldehyde, 5 mL glacial acetic acid and 0.1% DEPC. An RNA probe was generated from the *StZIP12* gene fragment amplified by PCR, labeled with 5-FAM, and added to the paraffin section made from previously prepared samples. The *StZIP12* probe sequence was 5′-CATCAGAGACCTGTGACTGCCCTTGTGCGACT-3′, and the probe concentration was used at 100 μmol/L.

Results

Genome-wide identification of *StZIP* genes

Twenty-nine StZIP genes were named StZIP1-29. The study shows that the minimum amino acid number of the *StZIP* gene family was 91 and the largest was 595, and its molecular weights were between 9.52 kDa and 61.74 kDa. Their isoelectric points (pIs) were predicted to range from 4.83 to 9.43 and *StZIP* were predicted to be localized in the plasma membrane, mitochondrial inner membrane, and endoplasmic reticulum membrane (Table 1).

Phylogenetic analysis of *StZIP* protein

In order to study the families of *StZIP*, using MEGA7.0, the phylogenetic tree of 29 amino acid sequences of *StZIP* domain was constructed by NJ method. The *StZIP* gene family was divided into three different groups, I to III (Fig. 1).

Chromosomal location and gene structure of *St*ZIPs

Using MapInspect tool, all *StZIPs* were located on 12 chromosomes, and the location map of *StZIP* was drawn. Twenty-nine *StZIP* was found in seven chromosomes out of twelve, and these genes were unevenly distributed on the chromosomes. Chromosome 2 had the largest number of *StZIP*, with 10 (about 34.4%), while chromosomes 5 contain only one *StZIP*. In addition, eighteen *StZIP* genes shared close physical distances on the chromosomes. The structural distribution of exons and introns of *StZIP* was obtained by analyzing the functional genome database of potato. The number of introns varied from 0 to 4. Out of the twenty-nine *StZIPs*, the shortest intron was in *StZIP16*, while the longest intron was in *StZIP22*. The number of exons of *StZIP* family members varies, consisting of 1–5 exons, of which the shortest exon was in *StZIP26* and *StZIP27*, while the longest exon was in *StZIP17*, *StZIP18* and *StZIP19* (Fig. 2).

Conserved motif analysis

The complete sequences of 29 StZIP proteins were used for protein sequence alignment and phylogenetic analysis. To further understand the evolutionary relationship and conserved motif of StZIP protein. According to Fig. 3, Twenty-nine StZIP proteins were divided into three subgroups, and 10 motifs were identified. therefore, we speculated that the subclass proteins in the same subgroup had similar conserved motif structure.

Cis-acting elements analysis in the promoter regions of *StZIP*

The potential cis-elements were analyzed by Plant Care database. The results showed that there were many plant

Table 1: Genome-wide identification of *StZIP* genes

Gene number	Gene ID	Amino acid	Molecular weight	PI	Subcellular localization
StZIP1	PGSC0003DMP400036679	360	38874.84	9.03	plasma membrane
StZIP 2	PGSC0003DMP400036678	258	28183.05	9.19	plasma membrane
StZIP 3	PGSC0003DMP400018321	91	9521.33	4.86	plasma membrane
StZIP 4	PGSC0003DMP400026275	132	14654.08	6.03	plasma membrane
StZIP 5	PGSC0003DMP400018277	281	30673.35	8.81	plasma membrane
StZIP 6	PGSC0003DMP400030981	354	38436.20	8.16	endoplasmic reticulum (membrane)
StZIP 7	PGSC0003DMP400030982	93	9783.61	5.92	plasma membrane
StZIP 8	PGSC0003DMP400018324	91	9539.36	4.86	plasma membrane
StZIP 9	PGSC0003DMP400026276	348	37239.86	6.50	plasma membrane
StZIP 10	PGSC0003DMP400018278	331	35691.60	8.48	plasma membrane
StZIP 11	PGSC0003DMP400020827	220	23667.77	7.07	plasma membrane
StZIP 12	PGSC0003DMP400038381	349	37258.65	5.40	plasma membrane
StZIP 13	PGSC0003DMP400023209	333	35589.76	6.05	plasma membrane
StZIP 14	PGSC0003DMP400023210	91	9529.25	4.83	endoplasmic reticulum (membrane)
StZIP 15	PGSC0003DMP400023211	340	36434.83	6.14	plasma membrane
StZIP 16	PGSC0003DMP400065179	120	12866.19	7.68	plasma membrane
StZIP 17	PGSC0003DMP400012145	595	61740.54	7.21	plasma membrane
StZIP 18	PGSC0003DMP400012146	595	61740.54	7.21	plasma membrane
StZIP 19	PGSC0003DMP400012144	535	55267.99	7.21	plasma membrane
StZIP 20	PGSC0003DMP400058248	216	23802.08	9.43	plasma membrane
StZIP 21	PGSC0003DMP400047196	328	35244.55	5.96	mitochondrial inner membrane
StZIP 22	PGSC0003DMP400042226	133	14739.45	3.96	plasma membrane
StZIP 23	PGSC0003DMP400042227	113	12262.79	9.61	endoplasmic reticulum (membrane)
StZIP 24	PGSC0003DMP400003849	334	36438.43	5.63	plasma membrane
StZIP 25	PGSC0003DMP400003848	316	34372.09	5.19	plasma membrane
StZIP 26	PGSC0003DMP400052150	407	43405.00	6.14	plasma membrane
StZIP 27	PGSC0003DMP400052151	284	30798.22	6.15	mitochondrial inner membrane
StZIP 28	PGSC0003DMP400052552	257	27678.64	6.29	plasma membrane
StZIP 29	PGSC0003DMP400052153	91	9566.36	6.51	plasma membrane

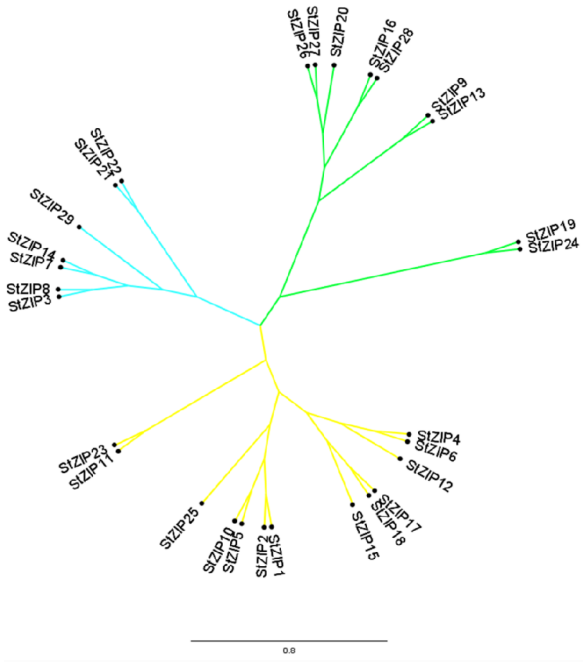

Fig. 1: Phylogenetic analysis of *StZIP* genes. A phylogenetic tree constructed using the neighborjoining (NJ) method in MEGA 7.0. Parameters were set as pairwise alignment, and 1000 bootstrap replicates. Branch lines with different colors indicated different subgroups. The proteins on the tree can be divided into three distinct subfamilies, which are indicated by different colored backgrounds

hormone action sites in the promoter region of *StZIP* gene. For example: Abscisic Acid (ABRE), MeJA responsiveness (TGACG-motif) and Salicylic Acid (TCA-element). Therefore, we speculated the potential role of *StZIPs* in development, stress adaptation and various hormone signaling pathways (Fig. 4).

Fig. 2: Chromosomal location and gene structure of ZIP. (**a**) Chromosomal distribution of *StZIP*. Vertical bars indicate locus of SotubMCs on potato chromosomes. Chromosome number is mentioned at the bottom of each chromosome. (**b**) The exon-intron structure of *StZIP* genes visualized by online tool GSDS 2.0, yellow boxes indicated exons and gray lines indicated introns

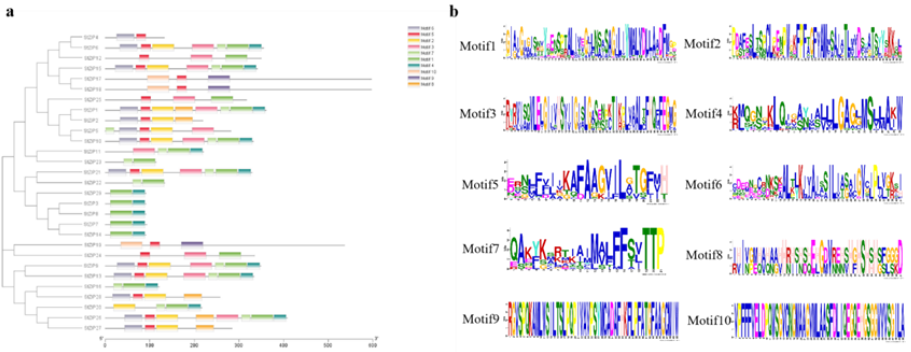

Fig. 3: Conserved motif analysis of *StZIP* genes. (**a**) The conserved motif of StZIP proteins analyzed by online program MEME server, different colored boxes indicated different motifs. (**b**) Highly conserved amino acid residues across all *StZIPs*

Conserved microsynteny of *StZIP* genes from two species

We identified some one-to-one micro collinearities between potato and tomato ZIP genes, nine pairs showed one-to-one microsynteny. For example,

ZIP9/Soly02g081600,ZIP6/Soly02g069190,ZIP5/Soly02g032100,ZIP21/Soly02g053370,ZIP25/Soly02g005260,ZIP15/Soly02g043200,ZIP12/Soly02g065380,ZIP26/Soly02g0065190,ZIP19/Soly02g087530 (Fig. 5).

Expression profile of *StZIP* genes in various organs and tissues

The expression data for *StZIPs* have been retrieved from RNASeq Expression Browser. Ten of the *StZIPs* (*StZIP1*, *StZIP4*, *StZIP11*, *StZIP15*, *StZIP16*, *StZIP20*, *StZIP21*, *StZIP22* and *StZIP24*) did not express in root and eleven of the *StZIPs* (*StZIP1*, *StZIP4*, *StZIP3*, *StZIP5*, *StZIP8*, *StZIP10*, *StZIP11*, *StZIP15*, *StZIP16*,

Fig. 4: Cis-acting elements analysis in the promoter regions of *StZIP*. ABRE, Abscisic acid responsive element; TGACG-motif, involved in MeJA responsiveness; TCA-element, Salicylic acid responsive element; TC-rich repeats, involved in defense and stress responsiveness; CGTCA motif, involved in Methyl-jasmonic acid (MeJA) response

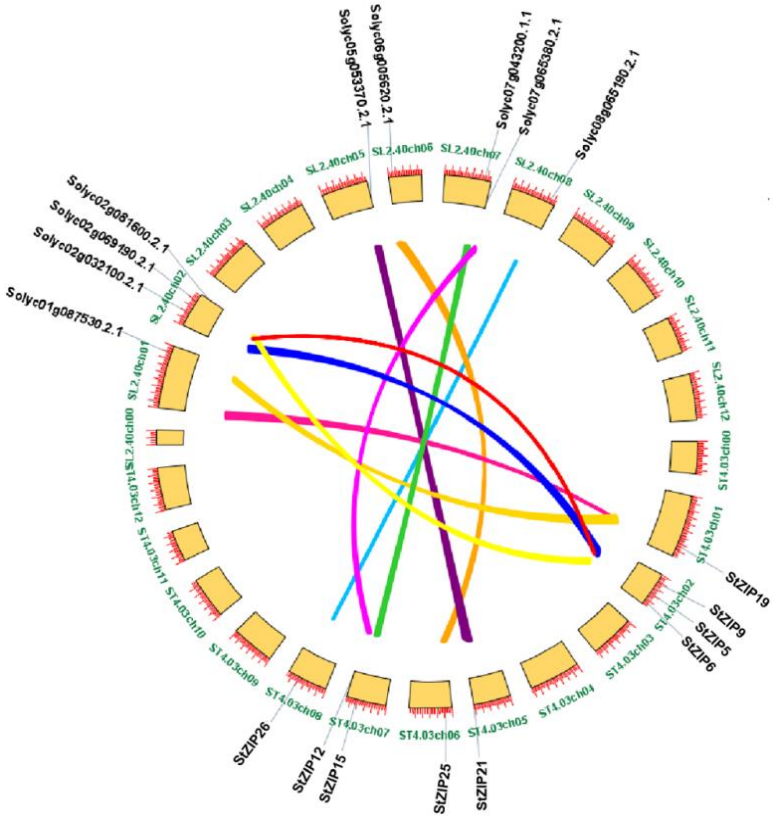

Fig. 5: Comparative orthologous relationships of *ZIP* from potato and tomato. Orthomcl was used to analyze the gene homology relationship between potato and tomato *ZIP* gene families, and Circos was used to visualize the gene chromosome localization and homology relationship. *ZIP* genes connecting potato genome and tomato genome are shown in colored links

StZIP20 and *StZIP24*) did not express in stem. On the contrary, *StZIP12*, *StZIP17* and *StZIP26* were expressed in different tissues and at different developmental stages (Fig. 6).

***StZIP12* expression patterns elicited by phytohormones**

The expression of *StZIP12* was up-regulated by the exogenous hormone ABA (Fig. 7). The relative expression

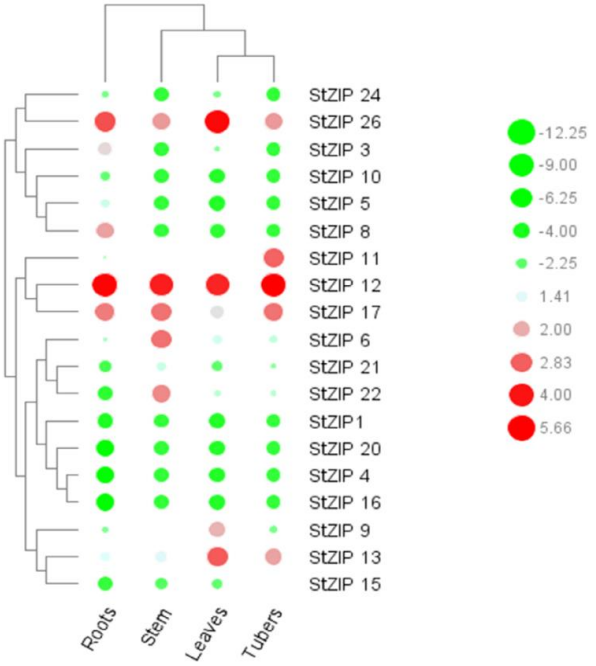

Fig. 6: Heat map showing expression profiles of *StZIPs* in different tissues. Heat-map showing expression patterns of *StZIP* in roots, stems, leaves and tuber based on RNA seq data. The Illumina RNA-seq data were reanalyzed, and the relative expression was calculated with respect to control samples

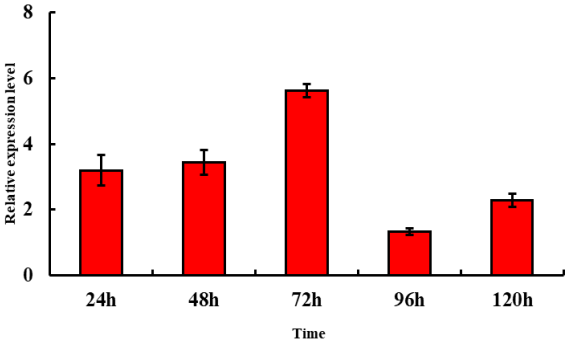

Fig. 7: The relative expression of *StZIP12* gene in potato seedlings treated with hormone ABA was analyzed by real-time quantitative PCR. Mean ± standard deviation (SD) (n=3 independent experiments, t-test)

of the gene reached the highest peak after 72 h of ABA treatment. However, the expression decreased significantly at 96 h, and began to increase at 120 h. ABA could strongly stimulate the up-regulated expression of *StZIP12* gene, indicating that the expression of *StZIP12* may play an important role in the process of hormone signal transduction.

StZIP12 expression levels induced by *R. solanacearum*

In investigate whether *StZIP12* responded to *R. solanacearum* treatment. The induced expression of *StZIP12* mRNA in potato seedlings inoculated with *R. solanacearum* was compared and analyzed by qRT-PCR method (Fig. 8). The results showed that after induction by *R. solanacearum*, the expression of *StZIP12* gene in the plant was smooth before 72 h, but increased 12-fold at 86 h, reached the highest level, and then gradually decreased at 96 h and 108 h. The expression level of *StZIP12* gene in plants is low when plants are not under stress. Under the stress of *R. solanacearum*, the expression of *StZIP12* gene in plants was up-regulated. Thus it could be seen that *StZIP12* gene was activated and expressed after infection with *R. solanacearum* and played an important role in potato resistance to bacterial wilt.

Tissue localization of *StZIP12* expression

Fluorescence in situ hybridization analysis showed that *StZIP12* mRNA was mainly distributed in the phloem of stem vascular system (Fig. 9a3) and leaf vascular bundle (Fig. 9c3). In addition, weak hybridization signals were observed in the control plants (Fig. 9b3 and 9d3). These results showed that *StZIP12* gene was located in vascular bundle and showed certain tissue specificity.

Discussion

First line indent analysis has been carried out in many model plants such as *A. thaliana*, *O. sativa and Z. mays.* There were sixteen ZIP family genes identified in *A. thaliana* (Milner *et al.* 2013), seventeen in *O. sativa* (Yang *et al.* 2009), and thirty-three in *Z. mays* (Xu *et al.* 2010). However, little was known about the potato ZIP (StZIP) gene family. Here, we identified and identified 29 *StZIP* genes (Table 1) and conducted comprehensive bioinformatics analysis (including chromosome mapping, phylogenetic analysis, gene structure, conserved motifs and cis elements in the promoter).

In this study, we identified 29 *ZIP* genes, which were divided into three groups in potato based on a comprehensive phylogenetic tree (Fig. 1). Phylogenetic analysis based on sequence alignments could be used as a rudimentary method to explore the molecular evolution of a gene family (Doxey *et al.* 2007), which meant that *ZIP* genes in the same clades may have similar functions. According to the result of MEME server, ten motifs were identified in *StZIP* proteins (Fig. 3). Expectedly, all of StZIP proteins contained a highly conserved ZIP domain (motif 1). By comparing the evolutionary tree and the conserved motifs of potato, we found that the conserved motifs of ZIP proteins changed when the potato ZIP protein evolved into a new family. With the occurrence of plant genome-wide events, genes were constantly evolving, mainly via appearance, loss and insertion of exons in genes. This research found that the numbers of exons in potato *StZIPs* were unequal, numbering 3–11 (Fig. 2b). After a series of comparisons, we found that *StZIPs* in the same subclade shared similar motifs composition and gene structures,

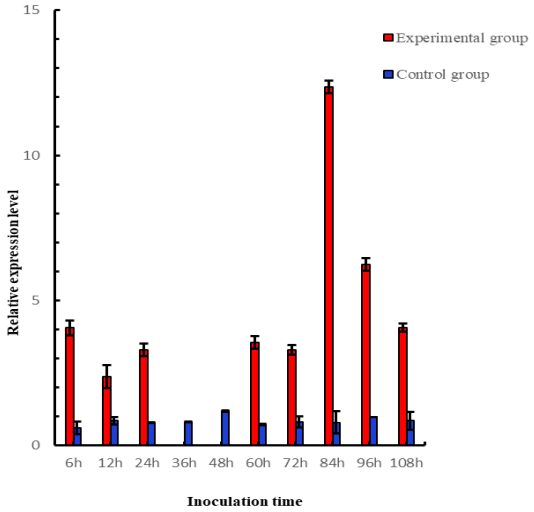

Fig. 8: The relative expression of *StZIP12* gene in potato seedlings inoculated with *R. solanacearum* species complex was analyzed by real-time quantitative PCR. Water was used as control. Normalization is carried out at each point in time based on the value of actin. The value is the average ± standard deviation (SD) (n=3 independent experiments, t-test)

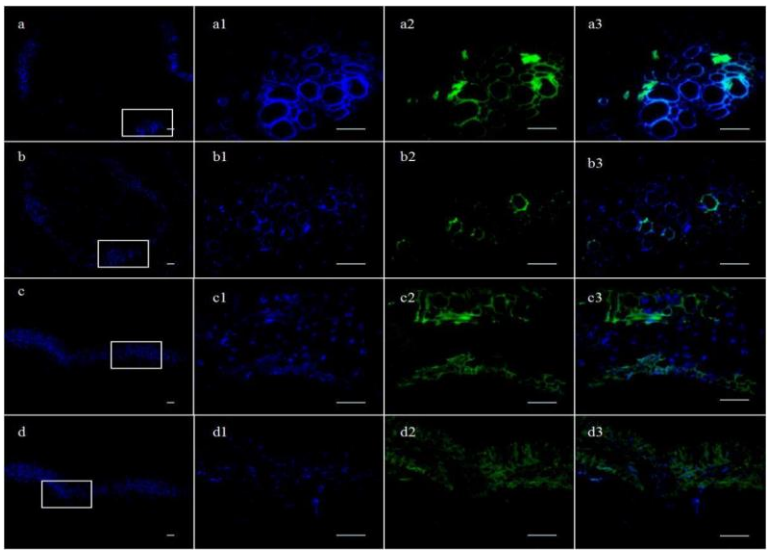

Fig. 9: *StZIP12* was located in the phloem and leaf vascular bundles of the stem vascular system. The stem and leaf tissues of potato seedlings were taken 48 h after inoculation with *R. solanacearum* species complex, and the localization of *StZIP12* in cells was observed by laser scanning imaging system. The first column and the second column are blue natural fluorescence, giving priority to the xylem, and the third column is green fluorescence, corresponding to the location of *StZIP12*. The fourth column is the combined image of the second column of blue

fluorescence and the third column of green fluorescence. (a-a3) Stems of potato seedlings inoculated with *R. solanacearum* species complex were crosscutting. (b-b3) Stems of potato seedling after water treatment were crosscutting. (c-c3) Leaves of otato seedlings inoculated with *R. solanacearum* species complex were crosscutting. (d-d3) Leaves of potato seedling after water treatment were crosscutting. The ruler is 20 μm

which implied that they may play similar molecular roles (Xu *et al.* 2019). The chromosome distribution analysis revealed that the 29 genes appeared clustered and scattered (Fig. 2a), 29 *StZIPs* were found on 7 potato chromosomes.

In addition, abiotic and biotic stresses and stress-related hormones, such as ABA, were found in the promoter region of the *StZIP* gene (Fig. 4). Plant hormone ABA played an important role in abiotic stress responses, especially in drought and saline stress (Tuteja 2007), and ABA was considered to be an important participant in plant immunity (Grant and Jones 2009). Therefore, we examined the response of *StZIP12* to the plant hormone ABA (Fig. 7). In addition, we also identified the biological function of *StZIP12*, which was regulating the resistance of potatoes to bacterial wilt (Fig. 8). Due to the interaction between *R. solanacearum* and plants in the potato seedling stage, in the

early stage of infection by *R. solanacearum*, the plant spontaneous immune system protected against the invasion of pathogens through a series of defense pathways. In the late stage of *R. solanacearum* infection, a large number of *R. solanacearum* gathered in the vascular system of stems and leaves, blocking the pathways of nutrients and water led to plant wilting to death (Peeters *et al.* 2013). At that time, plants mainly deal with water stress, and *StZIP12* participated in the ABA signal pathway to resist this water stress, which was the reason why *StZIP12* was significantly up-regulated after 60 h of induction by *R. solanacearum* and in the later stage of ABA induction. This was consistent with Wang's result (Wang *et al.* 2019). Therefore, we speculated that in addition to the role of *StZIP12* in plant growth and development. *StZIP12* genes were also involved in responses to abiotic and biotic stresses, such as plant hormones and pathogens.

By comparing RNA-seq data, we could further understand the tissue expression pattern of StZIP family genes. In this study, ten *StZIP* genes (*StZIP*-2, 7, 14, 18, 19, 23, 25, 27, 28 and 29) were not expressed in five potato tissues. These results suggested that these genes may be pseudogenes or expressed only in specific environmental conditions or developmental stages. In addition, the other nineteen *ZIPs* genes were expressed in five potato tissues. Among them, *StZIP8*, *StZIP12*, *StZIP17* and *StZIP26* genes have high expression in roots. *StZIP6*, *StZIP12*, *StZIP17*, *StZIP22* and *StZIP26* were extremely highly expressed in the stems. *StZIP12*, *StZIP13* and *StZIP26* had high expression in leaves, and *StZIP11*, *StZIP12*, *StZIP13*, *StZIP17* and *StZIP26* had high expression in tubers. It was further found that the expression of *StZIP* gene in different subfamilies had certain specificity, and the expression of *StZIP* gene in the same subfamily was significantly different in different tissues, indicating that it may have multiple functions in potato. Combined with the results of fluorescence in situ hybridization (Fig. 9), *StZIP12* was expressed in vascular bundles in a tissue-specific manner, and *R. solanacearum* also played a role in vascular bundles. Therefore, it was speculated that *StZIP12* gene played an important role in potato resistance to bacterial wilt. Bacterial wilt of potato was a disease of vascular bundle system. *R. solanacearum* played a role from the invasion of plant root to the vessel of xylem, and expands rapidly to the above ground part through the vascular bundle, resulting in the loss of the function of the vessel and further wilting of the plant, even death (Peeters *et al.* 2013). Compared with the control group, the expression of *StZIP12* gene induced by *R. solanacearum* was up-regulated when plants were stressed by *R. solanacearum* (Fig. 8).

Conclusion

In conclusion, through bioinformatics analysis of potato *ZIP* gene family, this study preliminarily explored the role of potato *ZIP* gene family in plant growth and development. In addition, the expression of *StZIP12* was altered by ABA and *R. solanacearum* treatments, indicating the transcription of *StZIP12* is controlled by a complex regulation network during interactions with pathogenic bacteria. In the future, the specific functions need to be further analyzed and verified by means of molecular biology.

Acknowledgements

This study is supported by the National Natural Science Foundation of China (31771858) and the 2020 Shanxi Excellent Graduate Innovation Project of China (2020SY321). Scientific and technological Innovation Project of School-level postgraduates of Shanxi normal University in 2019.

Author Contributions

Ruimin Yu performed primary experiments and and wrote the paper; Ruimin Yu and Yannan Chang analyzed the data; Tian Tian, Yanjie Song and Huanjun Wang was involved in the real-time PCR analysis; Gang Gao initiated and supervised the study and designed the experiments. All authors read and approved the final version of the manuscript

References

Assunção AG, H Schat, MG Aarts (2010). Regulation of the adaptation to zinc deficiency in plants. *Plant Signal Behav* 5:1553–1555

Bali S, BR Robinson, V Sathuvalli, J Bamberg, A Goyer (2018). Single Nucleotide Polymorphism (SNP) markers associated with high folate content in wild potato species. *PLoS One* 13; Article e0193415

Blaudez D, A Kohler, F Martin, D Sanders, M Chalot (2003). Poplar metal tolerance protein 1 confers Zinc tolerance and is an oligomeric vacuolar Zinc transporter with an essential leucine zipper motif. *Plant Cell* 15:2911–2928

Denancé N, P Ranocha, N Oria, X Barlet, MP Rivière, KA Yadeta, L Hoffmann, F Perreau, G Clément, A Maia-Grondard, GCV den Berg, B Savelli, S Fournier, Y Aubert, S Pelletier, BP Thomma, A Molina, L Jouanin, Y Marco, D Goffner (2013). Arabidopsis *wat1 (walls are thin1)*-mediated resistance to the bacterial vascular pathogen, *Ralstonia solanacearum*, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. *Plant J* 73: 225–239

Dereeper A, S Audic, JM Claverie, G Blanc (2010). BLAST-EXPLORER helps you building datasets for phylogenetic analysis. *BMC Evol Biol* 10; Article 8

Doxey AC, MWF Yaish, BA Moffatt, M Griffith, BJ McConkey (2007). Functional divergence in the *Arabidopsis* β-1,3-Glucanase gene family inferred by phylogenetic reconstruction of expression states. *Mol Biol Evol* 24:1045–1055

Gao S, Y Xiao, F Xu, X Gao, S Cao, F Zhang, G Wang, D Sanders, C Chu (2019). Cytokinin-dependent regulatory module underlies the maintenance of zinc nutrition in rice. *New Phytol* 224:202–215

Grant MR, JDG Jones (2009). Hormone (Dis) harmony moulds plant health and disease. *Science* 324:750–752

Grotz N, T Fox, E Connolly, W Park, ML Guerinot, D Eide (1998). Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc defificiency. *Proc Natl Acad Sci USA* 95:7220–7224

Guerinot ML (2000). The ZIP family of metal transporters. *BBA-Biomembr* 1465:190–198

He LY, L Sequeira, A Kelman (1983). Characteristics of strains of *Pseudomonas solanacearum* from China. *Plant Dis* 67:1357–1361

Kavas M, AK Kızıldoğan, B Abanoz (2017). Comparative genome-wide phylogenetic and expression analysis of SBP genes from potato (*Solanum tuberosum*). *Comput Biol Chem* 67:131–140

Kong CY, YP Luo, TT Duan, Z Xue, XD Gao, X Zhao, G Gao (2016). Potato remorin gene *strema4* cloning and its spatiotemporal expression pattern under *Ralstonia solanacearum* and plant hormones treatment. *Phytoparasitica* 44:575–584

Kozak K, A Papierniak, A Barabasz, M Kendziorek, M Palusińska, LE Williams, DM Antosiewicz (2019). *NtZIP11*, a new Zn transporter specifically upregulated in tobacco leaves by toxic Zn level. *Environ Exp Bot* 157:69–78

Lescot M, P Déhais, G Thijs, K Marchal, Y Moreau, DPY Van, P Rouzé, S Rombauts (2002). PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucl Acids Res* 30:325–327

Liang Y, N Wan, Z Cheng, Y Mo, B Liu, H Liu, N Raboanatahiry, Y Yin, M Li (2017). Whole-genome identification and expression pattern of the vicinal oxygen chelate family in rapeseed (*Brassica napus* L.). *Front Plant Sci* 8; Article 745

Milner MJ, J Seamon, E Craft, LV Kochian (2013). Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. *J Exp Bot* 64:369–381

Nussbaumer T, KG Kugler, KC Bader, S Sharma, M Seidel, KF Mayer (2014). RNA Seq Expression Browser-a web interface to browse and visualize high throughput expression data. *Bioinformatics* 17:2519–2520

Palmer C, M Guerinot (2009). Facing the challenges of Cu, Fe and Zn homeostasis in plants. *Nat Chem Biol* 5:333–340

Pang PX, L Shi, XJ Wang, YN Chang, YP Luo, JL Feng, H Eri, G Gao (2019). Cloning and expression analysis of the *StCUL1* gene in potato. *J Plant Biochem Biotechnol* 28:460–469

Pedas P, JK Schjoerring, S Husted (2009). Identification and characterization of zinc-starvation-induced zip transporters from barley roots. *Plant Physiol Biochem* 47:377–383

Pedas P, CK Ytting, AT Fuglsang, TP Jahn, JK Schjoerring, S Husted (2008). Manganese efficiency in barley: Identification and characterization of the metal transporter HvIRT1. *Plant Physiol* 148:455–466

Peeters N, A Guidot, F Vailleau, M Valls (2013). *Ralstonia solanacearum*, a widespread bacterial plant pathogen in the post genomic era. *Mol Plant Pathol* 14: 651–662

Peng WY, Z Ning, L Ting, YJ Wei, Z Xi, FC Xi, LS Gui, SH Jun (2019). Genome-wide identification and expression analysis of *StTCP* transcription factors of potato (*Solanum tuberosum L.*). *Comput Biol Chem* 78:53–63

Plaza S, KL Tearall, FJ Zhao, P Buchner, SP McGrathMcGrath, MJ Hawkesford (2007). Expression and functional analysis of metal transporter genes in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *J Exp Bot* 58:1717–1728

Ramesh SA, S Choimes, DP Schachtman (2004). Over-expression of an *Arabidopsis* zinc transporter in *Hordeum vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. *Plant Mol Biol* 54:373–385

Scheepers M, J Spielmann, M Boulanger, M Carnol, B Bosman, ED Pauw, E Goormaghtigh, P Motte, M Hanikenne (2020). Intertwined metal homeostasis, oxidative and biotic stress responses in the *Arabidopsis frd3* mutant. *Plant J* 102:34–52

Schmittgen TD, KJ Livak (2008). Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* 3:1101–1108

Song SW, LY Hao, P Zhao, Y Xu, NQ Zhong, HG Zhang, N Liu (2019). Genome-wide identification, expression profiling and evolutionary analysis of auxin response factor gene family in potato (*Solanum tuberosum* Group Phureja). *Sci Rep* 9; Article 1755

Thingholm, TE, L Rönnstrand, PA Rosenberg (2020). Why and how to investigate the role of protein phosphorylation in ZIP and ZnT zinc transporter activity and regulation. *Cell Mol Life Sci* 77:3085–3102

Thomas P, A Converse, HA Berg (2018). ZIP9, a novel membrane androgen receptor and zinc transporter protein. *Gen Compar Endocrinol* 257:130–136

Tuteja N (2007). Abscisic acid and abiotic stress signaling. *Plant Signal Behav* 2:135–138

Wang P, N Yamaji, K Inoue, K Mochida, JF Ma (2020). Plastic transport systems of rice for mineral elements in response to diverse soil environmental changes. *New Phytol* 226:156–169

Wang XJ, YP Luo, L Shi, PX Pang, G Gao (2019). Analysis of expression characteristics of Scarecrow-Like gene *Stsl-1* elicited by exogenous hormone and *Ralstonia solanacearum* infection in potato. *Intl J Agric Biol* 22:201–208

Xu YG, BS Wang, JJ Yu, GM Ao, Q Zhao (2010). Cloning and characterisation of *ZmZLP1*, a gene encoding an endoplasmic reticulum-localised zinc transporter in *Zea mays*. *Funct Plant Biol* 37:194–205

Xu Z, XZ Xuan, CL Chen, RZ Hai (2019). Comprehensive analysis of multiprotein bridging factor 1 family genes and *SlMBF1c* negatively regulate the resistance to *Botrytis cinerea* in tomato. *BMC Plant Biol* 19; Article 437

Yang X, J Huang, Y Jiang, HS Zhang (2009). Cloning and functional identification of two members of the *ZIP* (Zrt, Irt-like protein) gene family in rice (*Oryza sativa* L.). *Mol Biol Rep* 36:281–287

Zou Z, J Zhu, X Zhang (2019). Genome-wide identification and characterization of the Dof gene family in cassava (*Manihot esculenta*). *Gene* 687:298–307