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Por este medio presento respetuosamente como producción científica y humanística el artículo titulado **“The role of ZIP proteins in zinc assimilation and distribution in plants: current challenges”** en el **“Czech Journal of Genetics and Plant Breeding”** (Q3, IF: 1.277) el cual se enmarca en el Programa Nacional Estratégico **“Soberanía alimentaria”**.

Este trabajo coloca en perspectiva el conocimiento actual de los mecanismos de transporte de zinc hacia el interior de la planta, haciendo énfasis en los eslabones faltantes de conocimiento para un mejor aprovechamiento de esta información que se refleje en un mejor manejo de los cultivos, así como la producción de alimentos con mayor valor nutrimental.

Considero que este producto cumple con los requisitos para ser evaluado dentro de la convocatoria al Sistema Nacional de Investigadoras e Investigadores del CONAHCYT.

Agradezco de antemano su consideración a este producto, me despido cordialmente.

Atentamente

Dr. Juan Daniel Lira Morales

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The role of ZIP proteins in zinc assimilation and distribution in plants: current challenges

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Abstract: Soils with mineral deficiencies lead to nutritional imbalance in crops worldwide. Zinc (Zn) is a micronutrient that is fundamental for plant growth and development, being essential for the proper functioning of a range of enzymes and transcription factors. Zn transporters tightly regulate Zn homeostasis. Plants contain a large number of Zn-responsive genes that are specifically expressed under Zn deficiency to ensure the coordination of assimilatory pathways and meet the physiological requirements. This review brings together a range of studies that have been undertaken to investigate the effects of Zn status on the regulatory mechanisms involved in plant mineral nutrition. The ZIP (ZRT, IRT-like Protein) family is especially implicated in Zn transport and in the maintenance of cellular Zn homeostasis. Regulation of expression in relation to plant tissue, mineral concentration, and species has been determined for several ZIP family members. In the omic era, genomic and proteomic approaches have facilitated a rapid increase in our understanding of the roles of ZIP family members and their regulation, though significant knowledge gaps remain. A comprehensive understanding of ZIP proteins could lead to many potential molecular applications to improve crop management and food quality.

Keywords: homeostasis; IRT-like protein family; transcription; zinc-responsive genes; zinc transporters

Mineral nutrient acquisition has received considerable attention owing to its well-recognized importance in plant growth and development. All higher plants must coordinate the various pathways of nutrient absorption, transportation, and utilization. The element zinc (Zn) is essential in all organisms including the proper functioning of metabolism in plants because it is required for a wide range of vital plant processes, including CO₂ fixation during photosynthesis (SASAKI *et al.* 1998; HAJIBOLAND & AMIRAZAD 2010), protein synthesis (PRASK & PLOCKE 1971), maintenance of vacuolar homeostasis (LAN *et al.* 2013), nitrogen metabolism, and apoplastic signalling during controlled necrosis (SIEMIANOWSKI *et al.* 2013). Zn deficiency reduces the activities of carbonic anhydrase and superoxide dismutase; however, it triggers the production of ribonucleases, acid phosphatase, and peroxidase

(PANDEY *et al.* 2002; KOSAKAL & UNAL 2009). Approximately 50% of soils worldwide are deficient in Zn (WHITE & ZASOSKI 1999). Prominent among the adaptive responses of plants to Zn deficiency is the expression of Zn transporters.

Zinc homeostasis

Zn is taken up into plants mainly in the form of the bivalent cation Zn²⁺ from the growing medium. The uptake is mediated by membrane potential and Zn transporters are required to facilitate its diffusion across the cell membrane to the cytoplasm (EIDE *et al.* 1996; LEE *et al.* 2010; TIONG *et al.* 2015; OLSEN & PALMGREN 2014). Zn follows a symplastic pathway before being loaded into the xylem via active transport for long-distance movement (BROADLEY *et al.* 2007; OLSEN & PALMGREN 2014). The nature of Zn trans-

port mediated by importers and exporters makes it a suitable model for the coupled transcellular pathway recently proposed in plants (BARBERON & GELDNER 2014), although, more studies are needed for this assumption. The expression of Zn transporter genes has been detected in a range of tissues, and some studies have suggested that Zn transporters may also be involved in the transport of metals between plant organs, and/or in subcellular membrane transport (LÓPEZ-MILLÁN *et al.* 2004; CHEN *et al.* 2008; BASHIR *et al.* 2012). Several families of proteins have been shown to be necessary to meet the requirements for homeostatic adjustment: these include metal tolerance proteins (MTPs) that are involved in the storage of Zn in vacuoles (DESBROSSES-FONROUGE *et al.* 2005); heavy-metal ATPases (HMAs), which are involved in plant tolerance to heavy metals and also in the transport of Zn to the vacuole (MOREL *et al.* 2009); Natural Resistance Associated Macrophage Protein 4 (NRAMP4) which performs a similar function (OOMEN *et al.* 2009) and is involved in the root-shoot translocation of Zn (VERRET *et al.* 2004); the yellow stripe-like (YSL) family of proteins which are associated with Zn transport to reproductive tissues and leaves (WATERS *et al.* 2006); and Plant Cadmium Resistance2 (PCR2) which is associated with the long-distance transport of Zn (SONG *et al.* 2010). The introduction of Zn into roots is facilitated by nicotianamine, which forms complexes that are then delivered to the vascular tissues (DEINLEIN *et al.* 2012). Further information regarding Zn ligands and binding proteins, such as phytosiderophores, histidine, and glutathione, among others, as well as details of transporter proteins including MTPs and ZIF1 Like (ZIFL; Zinc-Induced Facilitator), can be found in SINCLAIR and KRAMER (2012); and the specific roles of each of these classes during the complete life cycle of plants are reviewed by OLSEN and PALMGREN (2014). ZIP (ZRT, IRT-like proteins) transporters comprise one of the principal mechanisms for the introduction and redistribution of Zn and other minerals (GROTZ *et al.* 1998), functioning throughout all organs (LI *et al.* 2013) and at all developmental stages (GAINZA-CORTES *et al.* 2012).

In *Arabidopsis thaliana*, ZIP1, ZIP2, ZIP3, ZIP5, ZIP6, ZIP9, ZIP10, and ZIP12 are involved in the uptake and translocation of Zn from the soil to the roots; ZIP1, ZIP2, ZIP3, ZIP4, ZIP5, ZIP7, ZIP9, ZIP10, ZIP11, and ZIP12 are expressed in shoots; and ZIP4, ZIP5, ZIP6, and ZIP9 are expressed in leaves. Together, therefore, these ZIP factors are involved

in the exchange of Zn throughout the principal organs of the plant. The genes encoding these ZIP factors are all responsive to Zn deficiency, except for ZIP6, which shows no change in transcript level for any tissue analysed (GROTZ *et al.* 1998; WINTZ *et al.* 2003; MILNER *et al.* 2013). ZIP1 is found in the vacuole and regulates the remobilization of Zn from this organelle, whereas ZIP2 is located in the root stele, where it functions in the transport of Zn to the xylem parenchyma (MILNER *et al.* 2013). Detailed histological analyses are required to establish the relationships between the various ZIP proteins – for example, to determine which of them show redundancy, and how they are expressed in different tissue layers. Overall, it is necessary to establish a detailed picture of the pathway of Zn movement throughout the plant.

Characteristics and regulation of the ZIP protein family

The predicted membrane topology of ZIP proteins (Figure 1a) is similar to that of most Zn transporter proteins. Their amino- and carboxy-terminal ends are located outside the plasma membrane, they have 6–9 putative transmembrane domains (TM), and they are 355–490 amino acids in length; the variation is largely because of a variable region located between TM-3 and TM-4. A variable loop is found in many Zn transporters and is suggested to serve as a metal-binding domain rich in histidine residues, which are thought to be cytoplasmic, though the exact role of this motif is unconfirmed. One of the key features of the Zn transporter proteins is positioned within the highly-conserved region TM-4, which is predicted to form an amphipathic helix with a fully-conserved histidine residue (EIDE *et al.* 1996; ZHAO & EIDE 1996; GROTZ *et al.* 1998; GUERINOT 2000; PENCE *et al.* 2000; ASSUNÇÃO *et al.* 2001; MOREAU *et al.* 2002; BURLEIGH *et al.* 2003; LÓPEZ-MILLÁN *et al.* 2004; MIZUNO *et al.* 2005; YANG *et al.* 2009; GAINZA-CORTES *et al.* 2012; LI *et al.* 2013). Substantial efforts have led to a better understanding of the interaction between Zn and the secondary structure of this family of proteins. For example, between TM-2 and TM-3, a specific sequence Ac-(95)MHVLPDSFEMLSICLEENPWHK(117)-NH₂ has a high affinity for Zn in IRT1 of *A. thaliana* (POTOCKI *et al.* 2013).

The expression of a number of ZIP family members is regulated by transcription factors of the basic-

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region leucine zipper (bZIP) family, which contain the 40–80 amino acid bZIP domain. Members of the bZIP family contain two structural regions; the first comprises a specific DNA binding site and an N-x7-R/K motif that interacts with the *cis*-regulatory elements of the target sequence (ASSUNÇAO *et al.* 2013), and the second is a leucine (Leu) zipper dimerization region (JAKOBY *et al.* 2002), exemplified in the homologs bZIP19 and bZIP23, which acts as a dimer to activate the expression of ZIP genes (ASSUNÇAO *et al.* 2010). Proteins with a high degree of similarity to bZIP19/23 (ASSUNÇAO *et al.* 2010) have been detected in other species besides *A. thaliana*, but further investigation is required to determine their role. For instance, in tomato (*Solanum lycopersicum*), *SlbZIP19/23* showed overexpression under Zn deficiency, similar to the overexpression of ZIP proteins (PAVITHRA *et al.* 2016). The transcription factor bZIP19/23 binds to a zinc deficiency response element (ZDRE) with the sequence 5'-RTGTCGACAY-3', which has been found

in the upstream region of several genes responsive to Zn deficiency (Figure 1b); however, a link still needs to be established between the location of this *cis* element in the promoter region of the gene and its expression under Zn deficiency. In this regard, in *A. thaliana*, the genes *ZIP4*, *ZIP9*, and *ZIP12* all contain the ZDRE and are upregulated under Zn deficiency, but the genes *ZIP1*, *ZIP3*, *ZIP5*, and *ZIP10* also contain the ZDRE element and yet are not responsive to Zn deficiency. Furthermore, *ZIP2* responds to Zn deficiency in spite of the absence of the ZDRE sequence, whereas in contrast *ZIP6* and *ZIP11* are non-responsive (ASSUNÇAO *et al.* 2010; JAIN *et al.* 2013). The basis of such divergent behaviour is difficult to unravel. To date, extensive analyses have been unable to identify a specific region or motif directly related to the expression of ZIP genes (JAIN *et al.* 2013) and the bZIP19/23 model proposed for the regulation of these genes still needs to be confirmed.

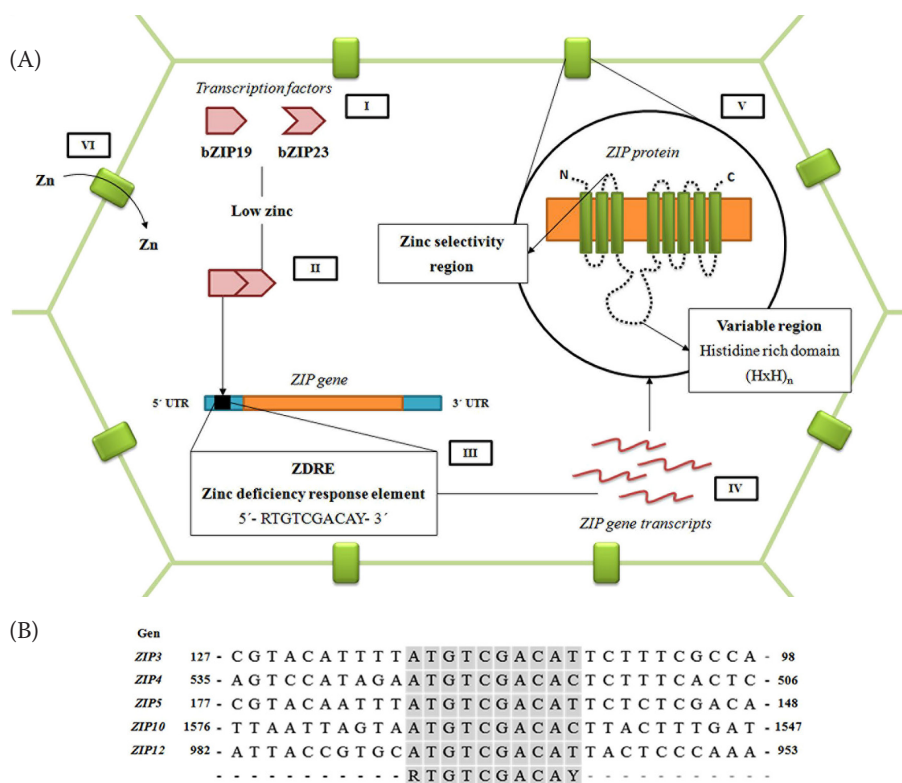


Figure 1. Schematic representation of the proposed ZIP protein regulation pathway (A): I – under normal levels of intracellular Zn, the bZIP19 and bZIP23 proteins remain separated; II – under Zn deficiency, bZIP19 and bZIP23 dimerize together to regulate the transcription of ZIP genes; III – bZIP19/23 recognizes the *cis* element ZDRE (Zn deficiency response element) present in some genes that are members of the ZIP family; IV – induction of transcription of a range of ZIP genes, leading to V – production of transmembrane proteins capable of VI – cellular uptake of Zn to maintain intracellular homeostasis; the ZDRE element (grey highlight) present in some of the ZIP genes of *A. thaliana* (B); numbers indicate locations upstream of start codons

The expression of the ZIP protein family in plants

Early investigations indicated that the homeostatic regulation of Zn may be significantly reduced under several environmental conditions, thus inducing the expression of ZIP proteins to achieve the required mineral acquisition. Interestingly, these transporters present a highly-regulated expression pattern as demonstrated in the model plant *A. thaliana* where the availability of genome sequences and knockout mutants has served for extensive characterization and classification of ZIP proteins of other species (SIVASUBRAMANIAN *et al.* 2015). To date, 18 members of the ZIP family of proteins have been identified in *A. thaliana*: 12 are ZIP (GROTZ *et al.* 1998; JAIN *et al.* 2013), three are IRTs (EIDE *et al.* 1996; VERT *et al.* 2001), and the remainder are AtIAR1 (LASSWELL *et al.* 2000), ZTP29 (WANG *et al.* 2010), and AtPutZnT (WANG *et al.* 2008). The evidence to date suggests a role for these proteins in the homeostasis of Zn in *Arabidopsis* and their upregulation under Zn deficiency. This view is based on observations of overexpression of transcripts encoding ZIP proteins, in some cases representing a 200-fold change in expression, in both roots and shoots (GROTZ *et al.* 1998; JAIN *et al.* 2013). Likewise, for IRT proteins, mRNA expression in both roots and shoots was induced under iron starvation (EIDE *et al.* 1996; VERT *et al.* 2001; LIN *et al.* 2009).

The interest in Zn nutrition is at the level of root absorption and the studies of ZIP proteins have been focused on the rhizosphere (for uptake) and shoot (for distribution) systems. In leaves (as distinct from shoots), ZIP genes are similarly upregulated under Zn deficiency like occurs in root and shoot, which reveals the integrated participation of these proteins in the Zn assimilation and redistribution in plants (LÓPEZ-MILLÁN *et al.* 2004). The understanding of specific expression patterns of ZIP genes and their role in plant adaptation to Zn deficiency would benefit the future development of improved approaches to plant mineral nutrition. Table 1 summarizes relevant evidences of expression patterns of ZIP family members over a range of species and tissues. Other predicted sequences not functionally characterized can be found in VATANSEVER *et al.* (2016).

The specific behaviour of ZIP member groups is complex and not well defined; this is due to the diversity of reports with respect to timing intervals, tissues analysed, nutrition level and even plant de-

velopmental stage as it defines the mineral requirements. This is why further research needs to go much farther under a structured design to improve the actual understanding of ZIP proteins, regulation mechanisms, role in metabolic pathways and future applications. While important information about homology and common evolutionary ancestors has been revealed from reliable strategies of similarity searching and sequence alignment, the most challenging question about similar regulation based on related sequences is complex and not answered. More functional studies are required to find a proper relationship or diversity in regulation-structure-function-localization characteristics.

Mycorrhizal fungi on ZIP proteins expression

The plant association with mycorrhizal fungi represents a mutual benefit that contributes to mineral uptake. Under Zn deficiency in soil, the fungal root colonization might be responsible for up to 24% of Zn uptake in tomato (WATTS-WILLIAMS *et al.* 2015). This contribution is reduced as the soil Zn concentration increases, representing a key symbiotic element of mineral absorption and inter-kingdom communication. Some evidences determined that the mycorrhizal symbiosis involves ZIP protein modulation. The expression of the gene for the zinc transporter MtZIP2, a protein localized in roots and stems of *M. truncatula*, was differentially regulated by *Glomus versiforme* colonization in conditions of Zn fertilization. MtZIP2 was upregulated in non-colonized roots exposed to toxic levels of Zn (100 mg/kg) but downregulated in colonized roots affecting Zn concentration in leaves (BURLEIGH *et al.* 2003). This finding suggests a positive influence of arbuscular mycorrhizae on mineral nutrition in the function of Zn concentration in soils. In *Hordeum vulgare* the colonization of *Rhizophagus irregularis* improved grain Zn concentrations at a low Zn level and the examination of five Zn transporters in roots confirmed the differential upregulation of HvZIP13 (WATTS-WILLIAMS & CAVAGNARO 2018). This regulatory mechanism opens a new re-assessment of ZIP gene expression extending their functional characterisation under mycorrhizal symbiosis.

Crop improvement prospects

As mentioned before Zn is an element required for the plant, but it is absent in half of the cultivable

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Table 1. Expression patterns of characterized members of the ZIP family of proteins

Name	Expression tissue				Reference
	root	leaf	shoot	other	
<i>Arabidopsis thaliana</i>					
ZIP1 ^{Mn,2^{Cu, Mn},3,4^{Cu},5^{Cu, Mn},9^{Mn},11,12}	▲		▲		EIDE <i>et al.</i> (1996); GROTZ <i>et al.</i> (1998); LASSWELL <i>et al.</i> (2000); HENRIQUES <i>et al.</i> (2002); WINTZ <i>et al.</i> (2003); WANG <i>et al.</i> (2008, 2010); LIN <i>et al.</i> (2009); NISHIDA <i>et al.</i> (2011); JAIN <i>et al.</i> (2013); MILNER <i>et al.</i> (2013); SHANMUGAM <i>et al.</i> (2013)
ZIP6 ^{Mn,10}	●		●		
ZIP7 ^{Mn,Fe}	▲		■		
IRT1 ^{Mn,Cd,Co,Fe,Ni}	▲				
IRT2 ^{Fe}	●				
IRT3 ^{Fe}	▲◆		▲◆		
AtIAR1					
ZTP29					
AtPutZnT					
<i>Arabidopsis hallieri</i>					
ZIP1,10	▲				BECHER <i>et al.</i> (2004); TALKE <i>et al.</i> (2006); LIN <i>et al.</i> (2009); SHANMUGAM <i>et al.</i> (2013)
ZIP3,9	●				
ZIP4,6	▲		▲		
ZIP12					
IRT1			▼		
IRT2 ^{Fe}					
IRT3 ^{Fe}	▲◆		▲◆		
<i>Glycine max</i>					
GmZIP1				▲ (nodule)	MOREAU <i>et al.</i> (2002)
<i>Hordeum vulgare</i>					
HvZIP1,2,3,5,7,8,10,13	▲		▲		PEDAS <i>et al.</i> (2008, 2009); TIONG <i>et al.</i> (2015)
HvZIP6,11,14	●		●		
HvZIP16	▲		●		
HvIRT1 ^{Mn,Fe}	●		●		
<i>Medicago truncatula</i>					
MtZIP1,3 ^{Fe} ,4 ^{Mn}	▲	▲			BURLEIGH <i>et al.</i> (2003); LÓPEZ-MILLÁN <i>et al.</i> (2004)
MtZIP2	▼		●		
MtZIP5 ^{Fe}	▲	●			
MtZIP6 ^{Fe} ,7 ^{Mn}	●	●			
<i>Oryza sativa</i>					
OsZIP1,2,3,5,6,7	▲		▲		BUGHIO <i>et al.</i> (2002); RAMESH (2003); ISHIMARU <i>et al.</i> (2005, 2006); CHEN <i>et al.</i> (2008); LEE <i>et al.</i> (2010)
OsZIP4	▲	▲	▲		
OsZIP8,9,13	●		●	■ (panicle)	
OsZIP10,12,16	ND		ND	ND	
OsZIP11	▲	▲		■ (panicle)	
OsZIP14			▲	ND	
OsZIP15	▲			■ (panicle)	
OsIRT1 ^{Fe}	●		●	■ (panicle)	
OsIRT2	●	●			
<i>Phaseolus vulgaris</i>					
PvZIP1,3,4,5,8,9,10,11,14,15,17,19	NT	NT		NT	ASTUDILLO <i>et al.</i> (2013)
PvZIP2,6,7,18	ND	ND		ND (pods)	
PvZIP12,16		▲		▲ (pods)	
PvZIP13		▲			

Table 1 to be continued

Name	Expression tissue				Reference
	root	leaf	shoot	other	
<i>Triticum turgidum</i>					
TdZIP1	▲				DURMAZ <i>et al.</i> (2010)
<i>Vitis vinifera</i>					
VvZIP1.1	ND	■	■	■ (fruit)	GAINZA-CORTES <i>et al.</i> (2012)
VvZIP2;5.1;6.1;8;13	■	■	■	■ (fruit)	
VvZIP3	■	■	■	▼ (fruit)	
VvZIP11.1	ND	■	■	ND	
<i>Zea mays</i>					
ZmZIP1 ^{Fe,2^{Fe}}	●		●	●	XU <i>et al.</i> (2010); LI <i>et al.</i> (2013)
ZmZIP3 ^{Fe}	▲◆		▲		
ZmZIP4 ^{Fe}			◆	▲ (embryo)	
ZmZIP5 ^{Fe}			▲◆	▲ (endosperm)	
ZmZIP6 ^{Fe}				▲ (embryo)	
ZmZIP7 ^{Fe}			◆	●	
ZmZIP8 ^{Fe}			▲◆	●	
ZmIRT1 ^{Fe}			▲▼	▲ (embryo)	
ZmZLP1	▲			● (flower)	
<i>Solanum lycopersicum</i>					
SlZIPL;3L;5		▲			ECKHARDT <i>et al.</i> (2001); PAVITHRA <i>et al.</i> (2016)
SlZIP2;4;5L1;5L2		●			
LeIRT1 ^{Fe,Mn,Cu}	■			■ (flower)	
LeIRT2 ^{Fe,Mn,Cu}	■				
<i>Noccaea caerulescens</i>					
NcZNT1	▲		▲		WU <i>et al.</i> (2009); MILNER <i>et al.</i> (2012)
TcZNT5 ^{Cd}	●		●		
TcZNT6 ^{Cd}	●		●		
<i>Thlaspi japonicum</i>					
TjZnt1 ^{Cd,Mn}					MIZUNO <i>et al.</i> (2005)
TjZnt2 ^{Mn}					

ND – not detected; ■ – detected, untested under Zn treatment; ▲ – upregulated under Zn deficiency; ● – detected, and unchanged under Zn starvation conditions; ▼ – upregulated under Zn excess; ◆ – downregulated under Zn excess; NT – candidate sequences found *in silico*, untested *in vivo*; superscripts indicate transporter affinity for other minerals: Mn – manganese; Fe – iron; Cd – cadmium; Co – cobalt; colours denote as follows: red – repression under deficiency; green – overexpression under deficiency; black – unavailable data

soils of the world (WHITE & ZASOSKI 1999). The knowledge of the specific transporter proteins of several minerals has led to novel strategies for crop management, particularly by plant transformation. Several studies have demonstrated the relationship between the overexpression of mineral transporters and the resistance to mineral deficiency (URAGUCHI *et al.* 2014; YAN *et al.* 2014); this relation identified the members of the ZIP proteins as potential tools for crop improvement and management, not only by the overexpression of members in plants, but also as potential molecular markers for the monitoring of the

crop nutrimental status (YANG *et al.* 2011). Studies provide striking information about the expression of these genes under mineral deficiency, showing that the same gene behaves in a similar manner even on different cultivars of the same species (YANG *et al.* 2011). This could be indicative of a conserved element over the evolutionary process, and this information can be used to extrapolate information to other members of the same genus or even family, but more detailed studies about the expression of these genes on many other cultivars of another species are necessary. But particularly as for Zn distribution improvement, this

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can be used not only as a strategy to translocate Zn from the soil to the cytosol, but also to improve the long-distance transport to sink sites, which can lead to improvement of plant processes and fruit quality (KENDZIOREK *et al.* 2014).

CONCLUSIONS AND PERSPECTIVES

The current challenges to achieve a better understanding of the ZIP family of Zn transporters, and of Zn homeostasis in general, can be summarized as follows: (1) identification of the sequence regions of Zn transporters that may be associated with higher affinity for Zn and/or higher rates of Zn uptake, so as to identify potential candidates with high Zn uptake rates for future crop-improvement applications; (2) determination of the region(s) in the promoter region and other *cis*-elements of ZIP genes that have the greatest influence upon transcription under Zn-induced stress; (3) extension of our current knowledge base to dicotyledonous crops of agricultural and economic relevance; and finally (4) identification of the specific role of each ZIP protein across different tissues, identifying ZIP proteins that are functionally redundant and those that are expressed constitutively under Zn deficiency.

Why should we focus research efforts on the ZIP family of Zn transporters? In answer to this question, we can highlight some promising future applications. First, the ZIP family proteins have a potential to be used as molecular markers of the nutrient status of crops (YANG *et al.* 2011); this can be realized through the use of molecular techniques such as qPCR to provide either relative or absolute quantification, or through novel approaches such as digital PCR, which offers the advantage of not requiring standards to achieve absolute quantification. On this basis, there is a prospect of developing faster and more precise molecular techniques for the early diagnosis of crop nutritional imbalances – especially important in the regions of the world that are directly dependent on agriculture. Effective management of the Zn or Fe status of crops is important in ensuring the supply of staple foods of high and consistent mineral nutritional quality to human and animal food chains. Secondly, the information obtained from in-depth histological and functional analyses in different crops, such as the roles of individual proteins and their specific uptake kinetics, will allow the selection of elite material for crop improvement through genetic modification (KENDZIOREK *et al.* 2014) or via traditional breeding

(or both). Thirdly, the study of these proteins has implications for food safety. For example, the consumption of high levels of Cd poses a risk of kidney toxicity in humans (YANG & SHU 2015); on the other hand, ZIP proteins are potential candidates for applications in bioremediation (ZHAO *et al.* 2003). In conclusion, the range of applications and benefits is clearly apparent, but to promote the exploitation of the ZIP family of Zn transporters in plants it is necessary to prioritize research in crop management, and in the enhancement of crop nutritional status and value.

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