

## Supporting Information

### **Effects of Deficiency and Excess of Zinc on Morphophysiological Traits and Spatiotemporal Regulation of Zinc-responsive Genes Reveal Incidence of Cross Talk Between Micro-and Macro-nutrients**

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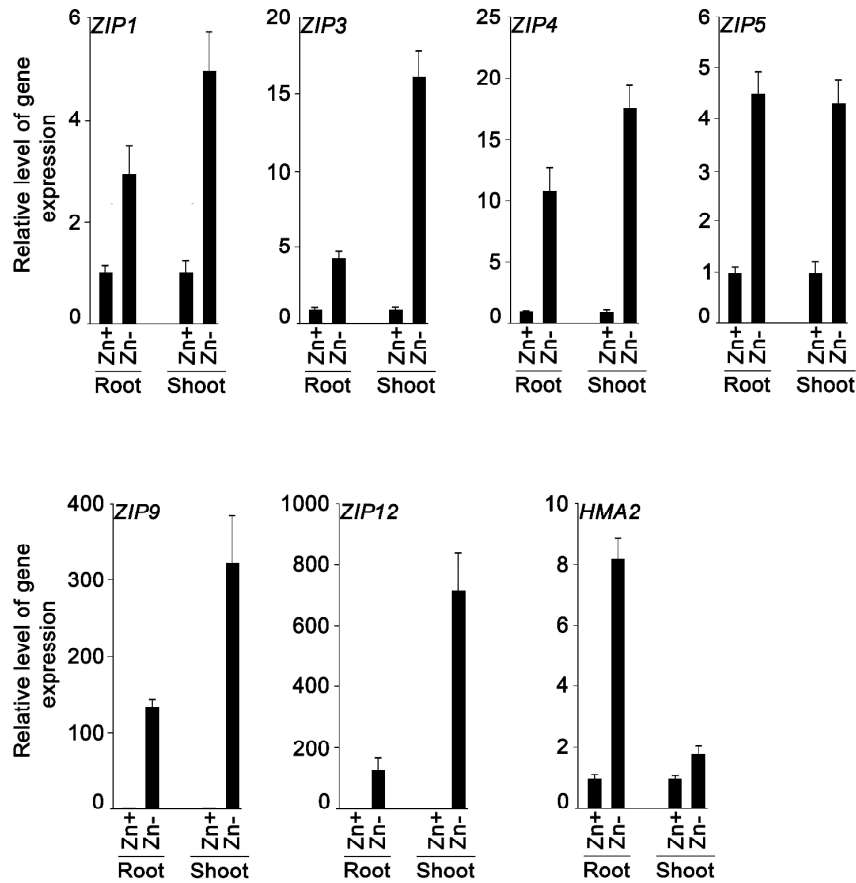
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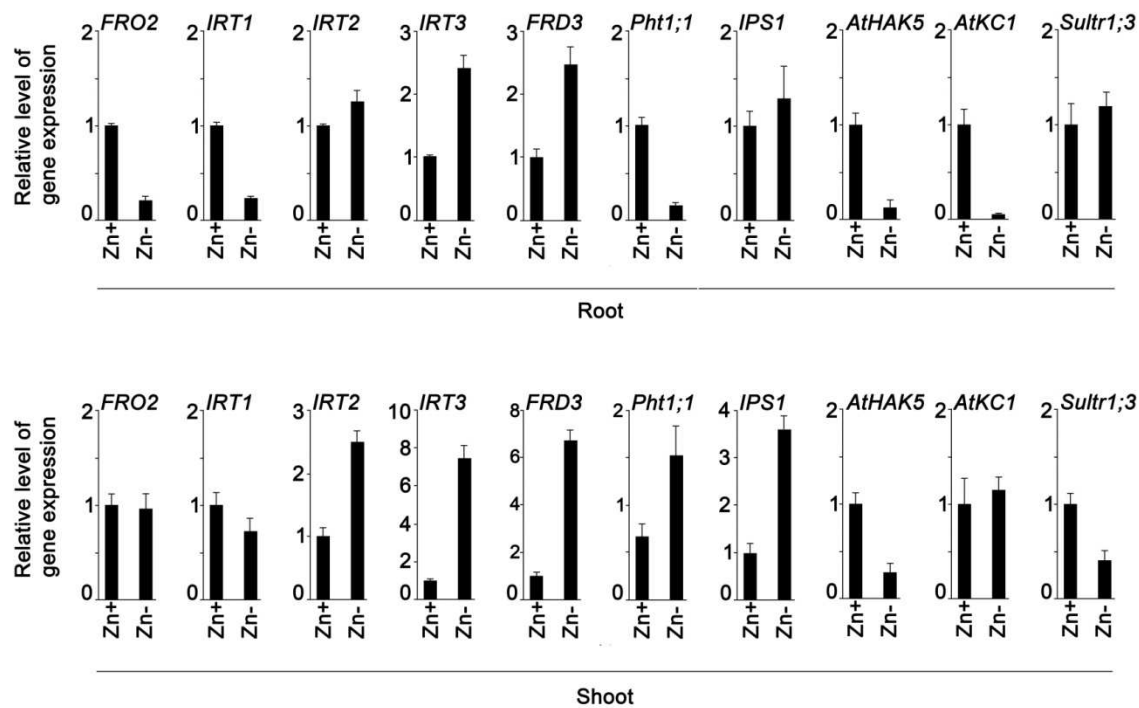
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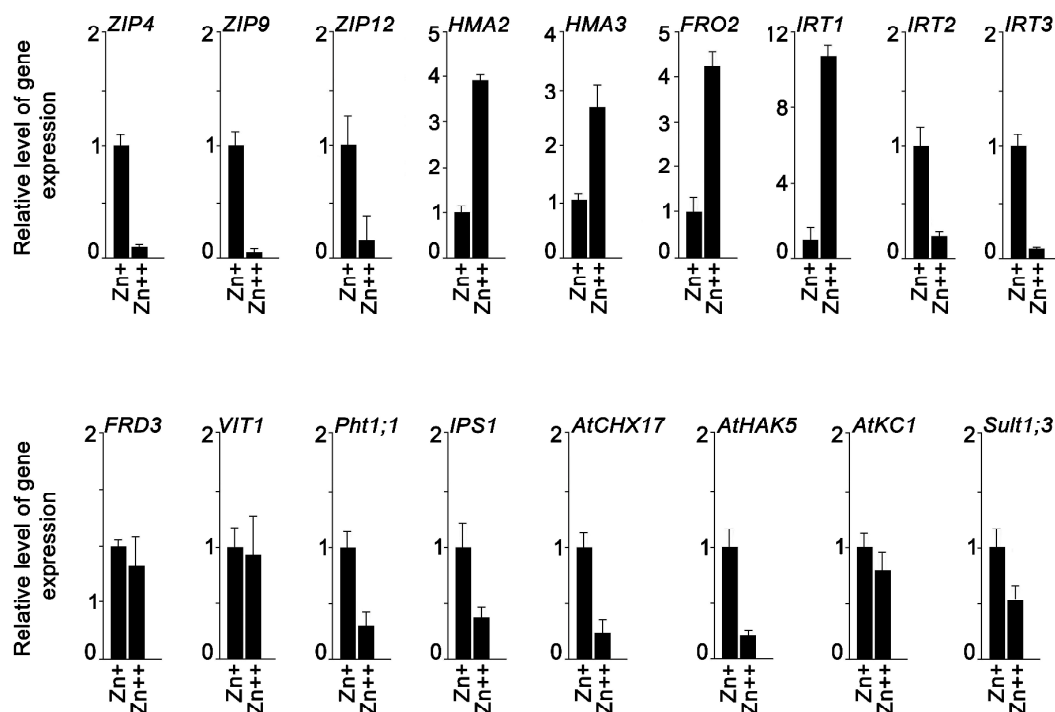
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**Figure S1.** Effect of long-term Zn deficiency on differential regulation of Zn-responsive genes. Real-time PCR analyses of the members of *ZIP* family and *HMA2* in roots and shoots of Arabidopsis seedlings grown hydroponically under Zn+ (3  $\mu$ M) and Zn- (0  $\mu$ M) conditions for 14 d. *At-ACT2* was used as an internal control, and Zn+ values were normalized to 1. Data presented are means of six technical replicates  $\pm$  SE. The relative level of the gene expression was considered significant when its value showed increase ( $>2.0$ -fold) in root and shoot under Zn- condition compared with their corresponding tissues grown under Zn+ condition.



**Figure S2.** Zn-deficiency affects regulation of genes involved in homeostasis of other nutrients. Real-time PCR analyses of Fe, P, K, and S responsive genes in roots and shoots of Arabidopsis seedlings grown hydroponically under Zn<sup>+</sup> (3  $\mu$ M) and Zn<sup>-</sup> (0  $\mu$ M) conditions for 7 d. *At-ACT2* was used as an internal control, and Zn<sup>+</sup> values were normalized to 1. Data presented are means of six technical replicates  $\pm$  SE. The relative level of the gene expression was considered significant when its value showed either increase (> 2.0-fold) or decrease (< 0.5) in Zn<sup>-</sup> roots and /or shoots compared with their corresponding Zn<sup>+</sup> tissues.



**Figure S3.** Excess Zn-mediated modulated molecular responses. Real-time PCR analyses of Zn, Fe, P, K, and S responsive genes in shoots of *Arabidopsis* seedlings grown hydroponically under Zn<sup>+</sup> (3  $\mu$ M) and Zn<sup>++</sup> (75  $\mu$ M) conditions for 7 d. *At-ACT2* was used as an internal control, and Zn<sup>+</sup> values were normalized to 1. Data presented are means of six technical replicates  $\pm$  SE. The relative level of the gene expression was considered significant when its value showed either increase (>2.0-fold) or decrease (< 0.5) in Zn<sup>++</sup> shoot compared with Zn<sup>+</sup> shoot.

**Table S1.** Primers used for Real-time PCR

Gene	AGI	Forward primer (5'→3')	Reverse primer (5'→3')
<i>ZIP1</i>	At3g12750	ATGTCTCTCCTCGCCATCTG	CGGCATTTGGTTTGTGTTGT
<i>ZIP2</i>	At5g59520	ACGTTGCGGTTAACCATCTC	CGAGGAAGACGGCAATAAAC
<i>ZIP3</i>	At2g32270	AGGTCTAGGCCTTGGTGGTT	CGATTCCCTATCGGTGTCGTT
<i>ZIP4</i>	At1g10970	CATCGCAGCTGATTTTCTGA	CCAGCTCCAAGGAACAACAT
<i>ZIP5</i>	At1g05300	TCGCAGCAGATTTTATGCAC	CCCTGCTCCAACAAGAAGAG
<i>ZIP6</i>	At2g30080	TGTGCTTAATGTTTCGCGGTA	GTTTCGGGTTCTGATCATCGT
<i>ZIP7</i>	At2g04032	AGGGATGGCCATACAAAAGA	TCAACAATCCTGCAGAGCAC
<i>ZIP8</i>	At5g45105	CTGCTCAATGCTTGTCTGC	AAGCTTGACGCTTCTTTGGA
<i>ZIP9</i>	At4g33020	ATTTGATCGCTGCGGATTT	TCATCCCAGCTCCAAGAAAC
<i>ZIP10</i>	At1g31260	CCTCGGCAGGACTACTCATC	AGCTTGATGCTCCTTTGCAT
<i>ZIP11</i>	At1g55910	CAACGACTCAAGGGTCCATC	TTTTGCCAACAAATGGTTCA
<i>ZIP12</i>	At5g62160	TGTTGATTTGGTAGCTCCACTTT	ACATCAAACCAGCACCAACA
<i>HMA1</i>	At4g37270	TGGTCGTGGAACAAGACATAG	CTGCTACTGGTTGGTTCTTGTG
<i>HMA2</i>	At4g30110	CTTGGTCAATGGTTTTTGTTC	ATGGCTATCGCTCTTCACTTTC
<i>HMA3</i>	At4g30120	TGTCAAGCCTGAACCTTGTG	TGAATGGCTCGCTATTGTTG
<i>HMA4</i>	At2g19110	ATCAGGCTTGGAAGTGAAGAAG	GTCTCTACCTTGACGGCTCTGT
<i>MTP1</i>	At2g46800	TGATGGAGAGTACACCCAGAGA	ACTTCTTCCATTTTCGAGCAAAC
<i>MTP3</i>	At3g58810	TTAATGGAGTCAACACCACGAG	CCACCACTTCTTCAATTTTACA
<i>PCR2</i>	At1g14870	ATATCGTAACCGCGGTGCT	TGCCTTAAACATTTCTGCTGTG
<i>AHA1</i>	At2g18960	CTGCAGCCAAAAGAAGATGTTA	TAGCTATCTCGGCCCTTCTCTT
<i>AHA2</i>	At4g30190	TGGAATCAGTCGTGAAGCTAAA	TGAGAGGATCGAAGATCAGTCA
<i>AHA7</i>	At3g60330	TTGCAGAATGAGAGAGCTTCAG	GATCATAGCCTTTCAGCTTTCG
<i>FRO2</i>	At1g01580	GTCCATCTCCAACATCTTCTCC	ATTCTTGTGGCGTACTTTCGAT
<i>IRT1</i>	At4g19690	CCCGCAAATGATGTTACCTT	ACTCGGTATCGCAAGAGCTG
<i>IRT2</i>	At4g19680	GATGGCATTCTTTTTCACTGG	TACTCGACAATGCGATTCCA
<i>IRT3</i>	At1g60960	ATGTTGTTTCCTAGGAGCTGGAC	CTAATTAAGCCCAAATGGCAAG
<i>FRD3</i>	At3g08040	GCGAGAGATTCTAGGCATCG	CCGACAAAAGCGGTATCAAT
<i>VIT1</i>	At2g01770	ACTTCCCTACATGCTCATACCG	CAAATATGAAGAGCGCAAACAG
<i>NRAMP3</i>	At2g23150	ACAACGTGTTTTCGCCAAAGG	ACCGCCTCCATATTTCTCCT
<i>NRAMP4</i>	At5g67330	ATCTCACGAGGACTCACGTACA	CATCATCCCTCTGTGGTTCTTT
<i>FER1</i>	At5g01600	CCACGGTACGAATCAAAACC	TCCTGCAAAGACAGAACAATG
<i>Phl1;1</i>	At5g43350	GAGCTCTAGGAAATGGCCGAAC	TGACAATCGCCGTGAAATGA
<i>Phl1;4</i>	At2g38940	TCGCTTATTGTGTTGGGTGT	TTTTACCCGGACATTTCTCTC
<i>IPS1</i>	At3g09922	GGTTCCTTCGGAATCAG	GAGTGGGTACAACCCAAACATAA
<i>RNS1</i>	At2g02990	TGGTTTCACTCCTTGGGTG	TTCGATTAAACCGGAACCAG
<i>At4</i>	At5g03545	TGTGAATGGAGCGATGAAGA	CTTGCCAAAGGATCGAAGTT
<i>PLDZ2</i>	At3g05630	CTACGATCGACCTTGGCATT	ATGGGAAGCACACAAGGTTC

<i>COPT1</i>	At5g59030	CCTCGCCTATCTAGTCATGCTC	TCCGAACAACATGAAACCAAC
<i>AtCHX17</i>	At4g23700	GGGAATTTGTTAACGGAAAGC	CGGACGGCTTGCTATATACTG
<i>AtHAK5</i>	At4g13420	ATCCGTGGAGGATCAAACAG	TGCCGTAATCTCGGTCTCTC
<i>AtKC1</i>	At4g32650	GATAACGGCGATCTTGCTTC	GTTGCCGTTGTTGTTGAGAA
<i>AtKEA5</i>	At4g32650	GATAACGGCGATCTTGCTTC	GTTGCCGTTGTTGTTGAGAA
<i>AtKUP3</i>	At3g02050	AGTTAACGGCGTCAAGTGGT	TATATGCAACACCCGCCTCT
<i>AtKUP12</i>	At1g60160	GCGTAGGGTCGTCTATGGAG	CCGAGTCTGTGGCTTCTCTC
<i>Sultr1;2</i>	At1g78000	CAACGCTGTGATTCCTTTGA	AGGCGATGAAATCGAGCTTA
<i>Sultr1;3</i>	At1g22150	AGGACAGCGATTCTTGAAAA	CGGAATCCTAGTCGCTTCAG
<i>Sultr3;4</i>	At3g15990	GTGAATCCTGTGGGGACTGT	AGTCCACTCAGACCCAATGC
<i>ACT2</i>	At3g18780	GATGAGGCAGGTCCAGGAATC	AACCCCAGCTTTTAAAGCCTTT

**TableS2.** The *cis*-regulatory elements in the promoter of *ZIPs*

Type of motif	Sequence	Function	No. of motifs occurrence					
			Responsive to Zn-			Non-Responsive to Zn-		
			4	9	12	6	10	11
Stress-responsive element								
HSE	AAAAAATTTC/ AGAAAATTGG	cis-acting element involved in heat stress responsiveness	2	1	3	0	0	0
MBS	CAACTG/CAACTG/ CGGTCA/TAACTG	MYB binding site involved in drought inducibility	2	2	0	0	0	0
EIRE	TTCGACC	elicitor-responsive element	0	1	1	0	0	0
ARE	TGGTTT	cis-acting regulatory element essential for the anaerobic induction	2	3	1	1	0	0
TC-rich repeats	GTTTTCTTAC/ ATTTTCTCCA/ ATTCTCTAAC	cis-acting element involved in defense and stress responsiveness	1	3	1	3	0	0
Box-W1	TTGACC	fungal elicitor responsive element	0	1	2	1	1	0
LTR	CCGAAA	cis-acting element involved in low-temperature responsiveness	0	0	0	1	0	0
Gibberellins-responsive element								
GARE-motif	AAACAGA/TCTGTTG	gibberellin-responsive element	7	2	3	0	0	0
Salicylic acid-responsive element								
TCA-element	CAGAAAAGGA/ CCATCTTTTT/ GAGAAGAATA	cis-acting element involved in salicylic acid responsiveness	3	2	1	0	1	0
Jasmonic acid-responsive element								
CGTCA-motif	CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness	1	2	1	3	2	0
TGACG-motif	TGACG		1	0	1	3	2	0
Ethylene-responsive element								
ERE	ATTTCAAA	ethylene-responsive element	1	0	3	0	0	0
Absciscic acid-responsive element								
ABRE	CGTACGTGCA/ TACGTG	cis-acting element involved in the absciscic acid responsiveness	0	2	0	2	0	0
Auxin-responsive element								
TGA-element	AACGAC	auxin-responsive element	0	1	1	0	0	1
AuxRR-core	GGTCCAT	cis-acting regulatory element involved in auxin responsiveness	0	0	0	1	0	0
Light-responsive element								
Box I	TTTCAAA	light responsive element	1	1	4	0	0	0
GAG-motif	AGAGATG/ GGAGATG/AGAGAGT	part of a light responsive element	0	3	1	1	0	1
I-box	CTCTTATGCT		1	1	2	0	0	0
LAMP-element	CTTTATCA		1	0	0	0	1	0
TCCC-motif	TCTCCCT		0	0	1	1	0	0
TCT-motif	TCTTAC		2	4	1	1	1	0
Chs-CMA2a	TCACTTGA		1	0	0	1	0	0
CAG-motif	GAAAGGCAGAC		1	0	0	0	0	0
CATT-motif	GCATTC		1	0	2	0	0	1
GA-motif	AAGGAAGA/ AAAGATGA		1	0	1	1	0	0
GATA-motif	GATAGGA/		0	2	1	1	0	0

	AAGATAAGATT								
LS7	CAGATTTATTTTT		0	0	0	1	0	0	
Gap-box	CAAATGAA(A/G)A		0	0	0	0	1	0	
GT1-motif	GGTTAA/GGTTAAT		0	1	0	1	0	0	
3-AF1 binding site	AAGAGATATTT	light responsive element	1	0	1	0	0	0	
Sp1	CC(G/A)CCC		0	0	1	0	0	0	
W-box	TTGACC		0	1	2	1	1	0	
MRE	AACCTAA	MYB binding site involved in light responsiveness	0	1	0	0	0	0	1
AE-box	AGAAACAT/ AGAAACAA	part of a module for light response	2	0	0	0	0	0	0
ACE	AAAACGTTTA	cis-acting element involved in light responsiveness	1	0	0	0	0	0	0
ATCC-motif	CAATCCTC		1	0	0	0	0	0	0
ATC-motif	AGTAATCT	part of a conserved DNA module involved in light responsiveness	0	0	0	1	0	0	
ATCT-motif	AATCTAATCT/ AATCTAATCC		0	0	0	1	0	0	1
Box 4	ATTAAT		0	1	1	1	1	1	1
<b>Tissue specific and other regulatory element</b>									
Skn_1-motif	GTCAT	cis-regulatory element involved in endosperm expression	1	5	2	3	1	1	
GCN4-motif	TGAGTCA		1	0	0	0	0	0	0
AAGAA-motif	GAAAGAA/ gGTAAAGAAA/	cis-element involved in seed specific expression	0	1	2	1	0	0	1
CCGTCC-box	CCGTCC	cis-acting regulatory element related to meristem specific activation	1	0	0	0	0	0	0
CAT-box	GCCACT	cis-acting regulatory element related to meristem expression	0	0	1	0	0	0	0
O2-site	GATGACATGG/ GATGACATGA/ GATGATGTGG	cis-acting regulatory element involved in zinc metabolism regulation	1	2	0	0	0	0	0
asl	TGACGTCA	cis-acting element involved in the root specific expression	0	0	0	1	0	0	0
AT-rich sequence	TAAAATACT	element for maximal elicitor-mediated activation (2 copies)	0	0	0	1	0	0	0
AT-rich element	ATAGAAATCAA	binding site of AT-rich DNA binding protein (ATBP-1)	0	0	0	1	0	0	0
3-AF3 binding site	CACTATCTAAC	Part of a conserved DNA module array (CMA3)	0	0	0	1	0	0	0
MSA-like	(T/C)C(T/C)AACGG (T/C) (T/C/A), TCAAACGGT	cis-acting element involved in cell cycle regulation	0	0	0	2	0	0	0
circadian	CAANNNNATC/ CAAAGATATC	cis-acting regulatory element involved in circadian control	0	0	4	3	3	1	



**Table S3.** *Arabidopsis thaliana* cis-regulatory element database was used for a comparative analysis of the predicted transcription factor binding site motifs in the promoters of *ZIP4*, *ZIP9*, and *ZIP12* that are induced significantly in Zn-deprived roots and shoots versus *ZIP6*, *ZIP10*, and *ZIP11* that do not show induction in roots and/or shoots during Zn deficiency

Name of the Binding Site	Binding Site Sequence	Binding Site Family/TF	Function	ZIP Promoters					
				Responsive to Zn-			Non-responsive to Zn-		
				4	9	12	6	10	11
W-box promoter motif	ttgact/tgacc	WRKY	Evidence for an important role of WRKY DNA binding proteins in the regulation of NPR1 gene expression	P	P	P (4)	P	P (2)	A
RAV1-A binding site motif	caaca	ABI3VP1	RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants	P	A	P (3)	P (4)	P (4)	P (4)
ATB2/AtbZIP53/AtbZIP44/GBF5 BS in ProDH	actcat	bZIP	A Novel Subgroup of bZIP Proteins Functions as Transcriptional Activators in Hypsolarity-Responsive Expression of the ProDH gene in Arabidopsis	p	A	P	A	A	P
LFY consensus binding site motif	ccactg/ccaatg	LFY	LFY controls the production of the flowers, which are formed in lieu of secondary inflorescences from the flanks of the shoot apical meristem	P	A	P	A	A	A
T-box promoter motif	actttg	---	Promoter analysis of the nuclear gene encoding the chloroplast glyceraldehyde-3-phosphate dehydrogenase B subunit of Arabidopsis thaliana	P	P	P	P (2)	P (2)	A
GATA promoter motif [LRE]	agatag/tgataa/a gataa/tgatag	---	Arabidopsis thaliana GATA factors: organization, expression and DNA-binding characteristics	A	P (3)	P (3)	P (6)	P (4)	P (4)
SORLIP1/2	agccac/gggcc	---	Identification of key promoter motifs involved in the network of light-regulated gene expression by combined analysis of genomic sequence and microarray data	A	P (4)	P	P (2)	A	P
MYB4 binding site motif	acctaac/accaaa c/aacaaac	MYB	Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses.	A	P (2)	P	P (2)	A	A
ARF/ARF1 binding site motif	tgtctc	ARF	Dimerization and DNA binding of auxin response factors	A	P	P	A	A	A
AtMYC2 BS in RD22	cacatg	BHLH	Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression	A	P	A	A	P	A

ATHB2 binding site motif	taataatta	HB	The Athb-1 and -2 HD-Zip domains homodimerize forming complexes of different DNA binding specificities	A	P	A	A	A	A
MYB binding site promoter	aacctaac	MYB	A flower-specific Myb protein activates transcription of phenylpropanoid biosynthetic genes	A	P	A	A	A	A
L1-box promoter motif	taaatgca	---	Identification of a cis-regulatory element for L1 layer-specific gene expression, which is targeted by an L1-specific homeodomain protein	A	P	A	A	A	A
TELO-box promoter motif	aaaccctaa	...	Plant interstitial telomere motifs participate in the control of gene expression in root meristems	A	A	P	A	P	A
CARG promoter motif	ccaataagg/cct tatttgg	MADS	Antagonistic regulation of flowering-time gene SOC1 by CONSTANS and FLC via separate promoter motifs	A	A	P (2)	A	A	A
Evening Element promoter motif	aaaatatct	...	Orchestrated transcription of key pathways in Arabidopsis by the circadian clock	A	A	P	A	A	A
I box promoter motif	gataag	---	An evolutionarily conserved protein binding sequence upstream of a plant light-regulated gene	A	A	A	P	P	A
DPBF1&2 binding site motif	acacttg	bZIP	Isolation of a novel class of bZIP transcription factors that interact with ABA-responsive and embryo-specification elements in the Dc3 promoter using a modified yeast one-hybrid system	A	A	A	A	P	P
SORLREP2/3	tgatatat	---	Identification of key promoter motifs involved in the network of light-regulated gene expression by combined analysis of genomic sequence and microarray data	A	A	A	P	A	P
MYB1 binding site motif	atcctacc	MYB	Isolation and characterization of a fourth Arabidopsis thaliana G-box-binding factor, which has similarities to Fosoncoprotein	A	A	A	A	A	P
ABRE-like binding site motif	gacgtgta	---	ABFs, a family of ABA-responsive element binding factors	A	A	A	P	A	A
BoxII promoter motif	gggttaa	---	Transcriptional activation by Arabidopsis GT-1 may be through interaction with TFIIA-TBP-TATA complex	A	A	A	P	A	A
RAV1-B binding site motif	caaca	ABI3VP1	RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants	A	A	A	P	A	A

A = Absent, P = present, and numerical in the parenthesis indicates the number of motifs

**Table S4.** Predicted subcellular localization of the 12 ZIPs in *Arabidopsis thaliana*

ZIP	Number of amino acids	Localization site	Prediction in Protein Databases with score					Number of trans-membrane segments	Position of GPI anchor
			Loc	ProtLoc	Pentamers	Neural Nets	Integral		
ZIP1	355	PM	PM/5	PM/3.3	PM/4.5	PM/2.7	PM/9.7	7	339
ZIP2	407	NL	0	0	PM/2.91	NL/2.7	NL/5.5	0	Nil
ZIP3	339	PM	PM/5	PM/3.3	PM/4.29	PM/2.7	PM/9.9	7	324
ZIP4	408	PM	PM/5	CL/1.7	CL/6.33	PM/2.04	PM/7.2	7	389
ZIP5	360	ER/CL	PM/5	PM/1.8	PM/4.94	PM/3	PM/9.7	7	Nil
ZIP6	341	PM	PM/5	CL/3.4	CL/4.56	ER/1.83	ER/4.97	0	Nil
ZIP7	365	PM	PM/5	PM/3.4	PM/4.3	PM/1.7	PM/9.3	6	346
ZIP8	299	PM	PM/5	PM/2	PM/4.34	PM/1.55	PM/9.3	4	284
ZIP9	344	PM	PM/0	PM/2.9	PM/4.35	PM/2	PM/9.8	6	325
ZIP10	364	PM	PM/5	PM/3.4	PM/4.53	PM/1.8	PM/9.5	7	356
ZIP11	326	PM	0	PM/5	PM/4.4	PM/1.7	PM/8.8	7	311
ZIP12	355	PM	PM/5	PM/3.4	PM/4.25	PM/2.7	PM/9.8	6	340

Plasma membrane (PM), Endoplasmic reticulum (ER), Nucleus (NL), Chloroplast (CL)

## **Materials and Methods**

**Plant Growth Conditions.** *Arabidopsis* (*Arabidopsis thaliana*) ecotype Columbia (Col-0) and transgenic *CycB1;1::uidA* were used in this study. Seeds were surface sterilized by treating with 70% (v/v) ethanol for 1 min, rinsed twice with sterile deionized water followed by constant shaking for 10 min in 50% (v/v) commercial bleach. Traces of bleach were removed by seven-eight washes with sterile deionized water. After 2 d of stratification at 4°C, surface-sterilized seeds were sown on polypropylene mesh (250 µm) and transferred to hydroponic set up as described for germination in one-half-strength MS medium with 1.5% (w/v) sucrose. To avoid entangling of the roots for subsequent root system architecture (RSA) analysis and histochemical study, 16 seeds were sown around the edges of the mesh. For collection of bulk tissue for ICP and Real-time PCR analyses, 100 seeds/mesh were sown. Hydroponic set up was maintained in a growth room under controlled conditions set at 16-h/8-h day/night cycle at 22°C with an average photosynthetically active radiation of 70 to 80 µmol m<sup>-2</sup> s<sup>-1</sup> provided by fluorescent tubes. In 5 d, roots of the germinated seedlings penetrated through the mesh and remained in contact with the nutrient medium in the Magenta (GA-7) box. Subsequently, mesh with germinated seedlings was removed from the Magenta box and washed twice with sterile deionized water and then twice with the nutrient solution to which they were to be transferred. Modified MS medium was used for preparing Zn<sup>-</sup> (0 µM), Zn<sup>+</sup> (3 µM), and Zn<sup>++</sup> (75 µM) and pH was adjusted to 5.7 with 0.5 M MES buffer. Seedlings were grown for 7 d, unless stated otherwise, under controlled conditions. For treatment with Cd, surface-sterilized seeds were sown at a density of 20-25 per Petri plate prepared with one-half-strength MS medium, 1.5% sucrose (w/v), and 1.2% (w/v) agar (Sigma A1296; lot no. 110K0195), and kept vertically oriented under controlled conditions in growth room for 2 weeks. Seedlings were pooled in a set of 5 each and their roots were passed through a hole made in the cap of 15 mL falcon tube and was screwed on to the falcon tube containing Zn<sup>+</sup> nutrient medium + 1.5% sucrose without Cd (Zn<sup>+</sup> Cd<sup>-</sup>) and with 10 ppm Cd (Zn<sup>+</sup> Cd<sup>+</sup>). Seedlings were treated with or without Cd supplemented medium for 3 d.

**Analysis of Morphological Traits.** Hydroponically grown seedlings on a mesh were removed from the Magenta box and transferred in an inverted position to a Petri plate containing water to facilitate the floatation of roots. Under stereomicroscope, roots were gently excised from the mesh at the hypocotyl-root junction and then transferred to 1% (w/v) agar plate. With a fine camel-hair brush, primary and secondary (first- and higher-order) roots were spread for revealing the architectural details of the root system. For documentation of total shoot area, leaves were dissected from the shoot, and transferred to an agar plate. Spread out roots and dissected shoots were then scanned in transmissive mode at 1200 dpi (HP scanner). Scanned images were used for documentation of different root traits and total shoot area using ImageJ (<http://rsb.info.nih.gov/ij/>) as described.

**Histochemical Analysis.** Roots of Zn<sup>+</sup> and Zn<sup>-</sup> *CycB1;1::uidA* seedlings grown hydroponically were incubated for 12 h at 37°C in a reaction mixture of 0.1 M Pi buffer (pH 7) containing 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, 5 mM K<sub>3</sub>Fe(CN)<sub>6</sub>, and 5 mM K<sub>4</sub>Fe(CN)<sub>6</sub>·3H<sub>2</sub>O. Stained roots were cleared with 70% ethanol. GUS expression in the primary roots of 10 to 15 samples for each of the treatments was captured using synchronoscopy (Olympus Vanox).

**Real-time PCR.** Total RNA was isolated from roots of Zn<sup>+</sup> Cd<sup>-</sup> and Zn<sup>+</sup> Cd<sup>+</sup>, and roots and shoots of the seedlings grown under different Zn regimes using RNeasy Plant Mini Kit (Qiagen; <http://www.qiagen.com/>). The total RNA isolated was treated with RQ1 RNase-free DNase

(Promega; <http://www.promega.com/>), and then 1 µg of the DNase-treated RNA was reverse transcribed using the SuperScript III first-strand synthesis kit (Invitrogen; <http://www.invitrogen.com/>). Real-time PCR was performed on Applied Biosystems 7500 real-time PCR system using SYBR Green detection chemistry (Applied Biosystems; <http://www.appliedbiosystems.com/>) and gene-specific primers. Actin (*ACT2*) was used as the internal control, and the relative expression levels of the genes were calculated by  $2^{-\Delta\Delta C_t}$  method of relative quantification. Products amplified using the real-time PCR primers were cloned in T-tailed vector pGMTEasy, sequenced using T7 polymerase universal sequencing primers and aligned the sequences with their corresponding original TAIR sequence submissions to confirm their identity. List of primers used for real-time PCR is given in Supporting Information Table S1.

**ICP analysis.** Hydroponically grown seedlings were washed thoroughly with sterile deionized water, blot-dried and then air-dried for 48 h in a hot air oven at 60°C. About 20 to 30 mg of the air-dried tissue samples were digested overnight with 3 mL conc. HNO<sub>3</sub> at 80°C in Pyrex tubes. Each digested sample was then diluted to 15 mL with sterile deionized water and analyzed on Thermo ICP spectrometer 6000. External standards procured from Inorganic Ventures ([www.inorganicventures.com](http://www.inorganicventures.com)) were used for running along with the digested tissue samples for comparison. ICP analysis was performed for estimating the concentration of macronutrients (P, K, S and Mg) and micronutrients (Fe, Zn, Mn and Cu) in the seedlings.

**Bioinformatic analyses.** The promoter sequences of *ZIP4* (1500 bp), *ZIP6* (1358 bp), *ZIP9* (1500 bp), *ZIP10* (887 bp), *ZIP11* (441 bp), and *ZIP12* (1500 bp) upstream of the annotated ATG start codon were obtained from The Arabidopsis Gene Regulatory Information Server (AGRIS; <http://arabidopsis.med.ohio-state.edu/>), and employed integrated database AtcisDB (The *Arabidopsis cis*-regulatory element Database) for the analysis of the putative transcription factor binding site motifs, and PlantCare (<http://oberon.fvms.ugent.be:8080/PlantCARE/>) for the analysis of *cis*-regulatory elements. Localization prediction of the ZIPs were done by submitting the amino acid sequences to [softberry.com](http://linux1.softberry.com/berry.phtml?topic=protcomppl&group=programs&subgroup=proloc) (<http://linux1.softberry.com/berry.phtml?topic=protcomppl&group=programs&subgroup=proloc>) which integrates results from 5 different protein databases.

**Statistical Analysis.** Statistical significance of the difference between mean values was determined using Student's t test. Different letters on the histograms are used to indicate means that were statistically different at  $P < 0.05$ .