Kasetsart J. (Nat. Sci.) 44 : 891 - 901 (2010)

Cloning and Molecular Characterization of the Zinc Transporter (*ZIP*) Gene from Cassava

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ABSTRACT

The zinc transporter (*ZIP*) involved in zinc uptake, controls the major steps in growth development tissue. In this study, cassava (*Manihot esculenta* Crantz var. HB80) *ZIP* cDNA of 726 bp length was isolated from developing fibrous roots during growth in tissue culture. This gene was highly homologous with those from other plant species. DNA blot analysis indicated that at least two copies of *ZIP* are present in the cassava genome. Three Thai cassava varieties, namely Huaybong 80 (HB80), Kasetsart 50 (KU50) and Rayong 1 (R1) were used to evaluate the steady-state transcript accumulation of transporter genes in a tissue culture experiment. Their agronomic characters were also observed. Total dry weight (TDW) of each variety was found to be highest after eight weeks culture with 0.15 mM $ZnSO_4.7H_2O$ added to the culture medium. The lengths of leaves, petioles, stems and fibrous roots were all found to be greatest with this treatment level.

A high differential expression of the *ZIP* gene in different tissue media was observed in the stems, fibrous roots and leafs, respectively. The results showed that the levels of *ZIP* genes were high in the treatment containing 0 mM and 0.15 mM of $ZnSO_4.7H_2O$, which was reflected by the highest fresh mass, dry mass, leaf number, fibrous root number, leaf length, stem length, petiole length and fibrous root length with the 0.15 mM $ZnSO_4.7H_2O$ treatment. The high level expression of these genes was most pronounced five to six weeks after harvest, because the plants used more nutrients at the start of growth development. The levels of these genes in R1 showed a higher level of expression than those of KU50 and HB80, reflected by R1 having the highest fresh mass when compared with KU50 and HB80.

Keywords: cloning, expression, nutrients uptake, zinc transporter, cassava

INTRODUCTION

Zinc (Zn) is an essential nutrient that plays important roles in numerous physiological processes in plants, serving as a cofactor for many enzymes and as the key structural motif in transcriptional regulatory proteins. Therefore, a deficiency of Zn decreases growth, but excess Zn has significant toxicity to biological systems through metal-based cytotoxic reactions. Thus, the uptake and transport of Zn must be strictly regulated. Intracellular Zn homeostasis is achieved through the co-ordinated regulation of specific transporters engaged in Zn influx, efflux and intracellular compartmentalization (Ishimaru *et al*., 2005).

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Received date : 02/03/10 Accepted date : 29/04/10

Plants have evolved mechanisms that allow the transport of metal ions, through different categories of metal transporters such as the *ZIP* (*ZRT*, IRT-like proteins) family (Guerinot, 2000), cation diffusion facilitators (CDFs) (Williams *et al*., 2000), heavy metal (or CPx-type) ATPases, the natural resistance-associated macrophage proteins (Nramps) and the cation antiporters (Gaxiola *et al*., 2002). They were found to be located in different organelles within the cell. *ZIPs* are involved in the transport of Fe, Zn, Mn and Cd with family members differing in their substrate range and specificity (Guerinot, 2000; Mäser *et al*., 2001). About 85 *ZIP* family members have now been identified from bacteria, archea and all types of eukaryotes, including 15 genes in *Arabidopsis thaliana* (Mäser *et al*., 2001). The *ZIP* proteins are predicted to have eight transmembrane (TM) domains with the amino- and carboxyl-terminal ends situated on the outer surface of the plasma membrane (Guerinot, 2000).

The first member of the *ZIP* family identified from a plant was *AtIRT1* (Iron-regulated transporter 1) (Eide *et al*., 1996), cloned from *A. thaliana* and identified by functional complementation of the Fe-uptake-deficient yeast double mutant *fet3 fet4*. *AtIRT1* is now thought to be the major transporter for high affinity Fe uptake by roots (Connolly *et al*., 2002; Vert *et al*., 2002). Plants overexpressing *AtIRT1* also accumulate higher concentrations of Cd and Zn than wild types under Fe-deficient conditions, indicating an additional role in the transport of these metals (Connolly *et al*., 2002). This is also supported by transport studies in tomato, where *LeIRT1* and *LeIRT2* genes were shown to be expressed in roots (Eckhardt *et al*., 2001). Expression of *LeIRT1* was found to be enhanced strongly by Fe limitation, but this was not the case for *LeIRT2*. *LeIRT1* was also up-regulated by P and K deficiencies in the root medium. This suggests a possible co-regulation of the transporter genes for certain essential minerals (Wang *et al*., 2002). Studies in yeast suggest that *LeIRT1* and *LeIRT2* also have a broad range of substrate transporters (Eckhardt *et al*., 2001). *OsIRT1* from rice, which has high homology to the *A. thaliana AtIRT1* gene, is also predominantly expressed in roots and is induced by deficiencies in Fe and Cu (Bughio *et al*., 2002).

ZIP1, *ZIP3*, and *ZIP4* from *Arabidopsis* restored Zn uptake to the yeast (*Saccharomyces cerevisiae*) Zn-uptake mutants, *Dzrt1* and *Dzrt2*, and have been proposed to play a role in Zn transport (Grotz *et al*., 1998; Guerinot, 2000). *ZIP1* and *ZIP3* are expressed in roots in response to Zn deficiency, suggesting that they may transport Zn from the soil to the plant, while *ZIP4* is expressed both in the roots and shoots, suggesting that it could transport Zn intracellularly and between plant tissues (Grotz *et al*., 1998; Guerinot, 2000). *ZIP2* and *ZIP4* rescued yeast mutants deficient in copper (Cu) transport and *ZIP4* is up-regulated in Cu-deficient roots (Wintz *et al*., 2003). *ZRT1* and *ZRT2* are high- and low-affinity Zn transporters, respectively (Eide, 1998; Guerinot, 2000). The proposed role of *ZIP* transporters in Zn nutrition has been supported further by the characterization of homologs from a number of plant species. For example, *GmZIP1* has been identified in soybean (Moreau *et al*., 2002), and functional complementation of *Dzrt1* and *Dzrt2* yeast cells showed that *GmZIP1* is highly selective for Zn, but not for iron (Fe) or manganese (Mn). *GmZIP1* is expressed specifically in nodules, but not in roots, stems or leaves, and the protein is localized to the peribacteroid membrane, suggesting a role in symbiosis. Ramesh *et al*. (2003) reported that *OsZIP1* and *OsZIP3* are also functional Zn transporters in rice (*Oryza sativa*) plants. To date, there has been limited reporting of molecular information about *ZIP* gene in cassava. In the current study, a *ZIP* cDNA from cassava fibrous root was cloned and characterized and its differential expression during growth development studied at various concentrations of $ZnSO_4.7H_2O$ in Murashige and Skoog (MS) medium.

MATERIALS AND METHODS

Plant materials

Cassava (*Manihot esculenta* Crantz cv. HB80 KU50 R1) tissue was grown in different MS media containing 0, 0.037, 0.075, 0.15 and 0.22 mM $ZnSO_4.7H_2O$, respectively. Other plant parts (young leaves, stems and fibrous roots) were obtained at weeks 4-8 after subculture. These plant tissues were immediately frozen in liquid nitrogen and kept at -80°C until use.

Total RNA extraction

Total RNA was extracted from the cassava tissue samples using the method of Salzman *et al*. (1999). RNA used for real-time polymerase chain reaction (RT-PCR) amplification was isolated from cassava fibrous roots at 5 week. RT-PCR analysis was performed in young leaves, stems and fibrous roots for 0, 0.037, 0.075, 0.15 and 0.22 mM $ZnSO_4.7H_2O$ treatments.

Isolation of *ZIP* conserved region cDNA

Cassava *ZIP* cDNA was isolated from fibrous roots using a PCR approach, based on conservation of zinc transporter coding sequences among plant species. A multiple sequence alignment of several previously reported plant *ZIP* cDNA sequences in GenBank was performed on *Glycine max, Fragaria x ananassa and Medicago truncatula*, using the ClustalW method (Chenna *et al*., 2003). The cDNA was synthesized from the adaptor-oligo (dT_{18}) primer, with 5 μg of total RNA from fibrous roots, using M-MuLV reverse transcriptase (Fermentas). PCR reactions were assembled with Dream Taq Polymerase according to the manufacturer's recommendation (Fermentas). An initial internal fragment of *ZIP* gene was amplified by using the forward primer ZpF 5′-TTGCCTGAAGGTTACGAGAG-3′ and the reverse primer ZpR 5′-CTCTTCTTGTT GGAGCA -3′. The 3′ end of the *ZIP* cDNA was amplified by using the forward gene-specific primer and the reverse oligodT M13R primer. The 5′ ATG of *ZIP*-coding sequence was amplified using the degenerated primer and the gene-specific primer. All PCR reactions were analyzed by electrophoresis. One single band of internal fragment from the 3′end and 5′ATG from PCR products were cut and cloned into the pGEM-T easy vector before sequencing. Five clones from each fragment were sequenced at 1st BASE, Malaysia. The BLAST analysis confirmed that five clones from each fragment were the expected *ZIP* gene. The sequences from the overlapping *ZIP* cDNA fragments were assembled into a contiguous sequence using the GeneDoc program.

Nucleotide sequence analysis

Sequence analyses were performed using the BLASTX program obtained from the National Center for Biotechnology Information (Available from: http://www.ncbi.nlm.nih.gov/genbank/ [cited 2010 January 9]). The phylogenetic relationship from the deduced amino acid sequences was constructed by the neighbor-joining method (Saitou and Nei, 1987) with 1,000 bootstrap replicates using the MEGA version 4 software program (Tamura *et al*., 2007). Comparisons of deduced amino acid sequences were performed using the GeneDoc program.

RT-PCR analysis

For RT-PCR analysis, about 500 ng of total RNA from other cassava tissue (young leaves, stems and fibrous roots) raised in different MS media (0, 0.037, 0.075, 0.15 and 0.22 mM $ZnSO_4.7H_2O$ treatments) were used.

The LightCycler® 480 system PCR run with LightCycler® 480 RNA Master Hydrolysis Probes, using a LightCycler® 480 Multiwell Plate 96 was performed. Using a LightCycler® 480 RNA Master Hydrolysis Probes kit, the reaction contained up to 500 ng of total RNA, 1μL of 10 mM of the specific primers (forward and reverse), 0.25μL of 10 nM of the specific probe, 0.7 μL of

activator, 0.5 µL of enhancer, 3.7 µL of LightCycler® 480 RNA Master Hydrolysis Probes and up to 10 µL of deionized water. The results were analyzed using LightCycler® 480 software (Roche), and compared using the comparative threshold T cycle C method according to the manufacturer's instructions for data normalization. The NADH-plastoquinone oxidoreductase subunit 5 was used as the gene internal control.

DNA gel blot analysis

For DNA gel blot analysis, genomic DNA was isolated from young leaves of cassava, based on a protocol described by Dellaporta *et al*. (1990). Approximately 20 µg of genomic DNA was digested with three restriction enzymes (*Sac*I, *Sal*I and *Pst*I), separated in a 1% agarose gel, and transferred to a nylon membrane (Schleicher & Schuell). The membrane was hybridized using the same probes used for RNA gel blot analysis for 16 h at 60°C in a hybridization buffer containing 5X SSC, 0.1% (w/v) N-lauroylsarcosine, 0.02% sodium dodecyl sulfate (SDS) and 2% blocking agent (Roche). The remaining procedures were similar to those used in the RNA blot hybridization.

RESULTS

Cloning, sequencing and characterization of *MeZIP* gene

The zinc transporter gene (*MeZIP*) was cloned from cassava fibrous root using primers designed to compass highly conserved regions of the *ZIP*. Sequencing revealed that the conserve *MeZIP* clone was 723 nucleotides in length (Figure1). The complete nucleotide of the *ZIP* sequence was 1,062 bp long, based on *Fragaria x ananassa*. The sequence was blasted and aligned by the program BLASTX and CLUSTALW. The deduced amino acid sequence of *MeZIP* was used to compare the amino acid composition of the polypeptide to the other plant *ZIP*s. The analysis revealed a high degree of sequence identity of 67-73% when compared to other plants. The phylogenetic tree indicated that the amino acid sequence of *MeZIP* was in separated clusters. *MeZIP* appeared in the cluster with a closer relationship to *Fragaria x ananassa ZIP* than to that of *Glycine max ZIP* (Figure 2).

***MeZIP* expression is dependent on the availability of Zinc in the medium**

To define the $ZnSO_4.7H_2O$ concentration at which the zinc transporter genes are expressed, cassava plants were grown in the presence of different concentrations of zinc ($ZnSO_4.7H_2O$) in MS media. After 4-8 week of treatment, the young leaves, stems and fibrous roots were harvested for isolation of total RNA. RT-PCR analyses were carried out to determine the expression of the *MeZIP* cDNA. The results in Table 1A show that R1 had higher transcript levels of *MeZIP* than those of KU50 and HB80. With regard to the expression pattern of *MeZIP* in various tissues of cassava during the vegetative stage, the *MeZIP* gene was highly expressed in young leaves and stems when compared with fibrous roots. Thus, the expression pattern of *MeZIP* demonstrated that this gene was differentially expressed in various tissues and at different growth stages.

As shown in Table 1B, the transcript level of the zinc transporters was high in the young leaves, stems and fibrous roots of plants grown at 0 and 0.15 mM $ZnSO_4.7H_2O$, and decreased with increasing zinc concentrations. *MeZIP* was more sensitive to decreasing zinc levels and the transcript level was significantly lower in the 0.22 mM zinc treatment. The regulation of *MeZIP* expression by zinc availability was further examined by resupplying $ZnSO_4.7H_2O$ to the plants that were zinc deficient and strongly expressing the genes. When 0.15 mM $ZnSO_4.7H_2O$ was resupplied to these plants, transcript levels of the gene increased when compared with the control (0.075 mM). These observations suggested the existence of a fine

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  1 TTGCCTGAAGGTTACGAGAGCTTGACATCCCCTTGTCTCAATGAGAATCCATGGGGAAAG
  1   L   P   E   G   Y   E   S   L   T   S   P   C   L   N   E   N   P   W   G   K
 61 TTTCCTTTCACTGGTTTTGTGGCCATGGTTTCTGCCATTGAGACTTTGATGGTTGATGCT
 21   F   P   F   T   G   F   V   A   M   V   S   A   I   E   T   L   M   V   D   A
121 TTTGCGACTTCTTATTATACCAAGTCTCATGGACAAGTCAGAAATATAGCTGGAGATGAG
 41   F   A   T   S   Y   Y   T   K   S   H   G   Q   V   R   N   I   A   G   D   E
181 GAGAAAACAGAAGAAGATGGAGGATTTCATACTCATGCAACTCATGATCATTCTCATTGT
 61   E   K   T   E   E   D   G   G   F   H   T   H   A   T   H   D   H   S   H   C
241 TCAGGTTTGATTGAGAATTCTGCTTCACCTGAACTCCTTCGCCATCGAGTTATTTCTCAG
 81   S   G   L   I   E   N   S   A   S   P   E   L   L   R   H   R   V   I   S   Q
301 GTTTTGGAGTTGGGAATTGTGGTTCACTCTGTGATAATAGGAATCTCTTTAGGTGCTTCT
101   V   L   E   L   G   I   V   V   H   S   V   I   I   G   I   S   L   G   A   S
361 CAAAGTCCTAAAACAATAAGGCCTCTAGTAGCTGCGCTCACCTTTCATCAGTTCTTTGAG
121   Q   S   P   K   T   I   R   P   L   V   A   A   L   T   F   H   Q   F   F   E
421 GGTATGGGACTTGGTGGTTGCATTTGTCAGGCAAAATTTAAGGGAAGAGTTATGGCGATT
141   G   M   G   L   G   G   C   I   C   Q   A   K   F   K   G   R   V   M   A   I
481 ATGGCACTTTTCTTCTCTCTGACAACACCAATTGGGATTGGGATTGGTATTGGGATATCA
161   M   A   L   F   F   S   L   T   T   P   I   G   I   G   I   G   I   G   I   S
541 AACGCGTACAATGAAAACAGCCCAACTGCCCTAATTGTTGAAGGGATTTTTAATTCAGCC
181   N   A   Y   N   E   N   S   P   T   A   L   I   V   E   G   I   F   N   S   A
601 TCAGCTGGAATTTTAATTTACATGGCATTGGTGGATCTTCTTGCTGCTGATTTCATGAAT
201   S   A   G   I   L   I   Y   M   A   L   V   D   L   L   A   A   D   F   M   N
661 CCCAAAGTACAGGTCAATGGAAAACTTCAAATTGGAGTTAATCTTTCTCTTCTTGTTGGA
221   P   K   V   Q   V   N   G   K   L   Q   I   G   V   N   L   S   L   L   V   G
721 GCA
241   A
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Figure 1 Nucleotide and amino acid sequence of a partial *MeZIP* clone. Numbers on the left refer to nucleotide and amino acid residues.

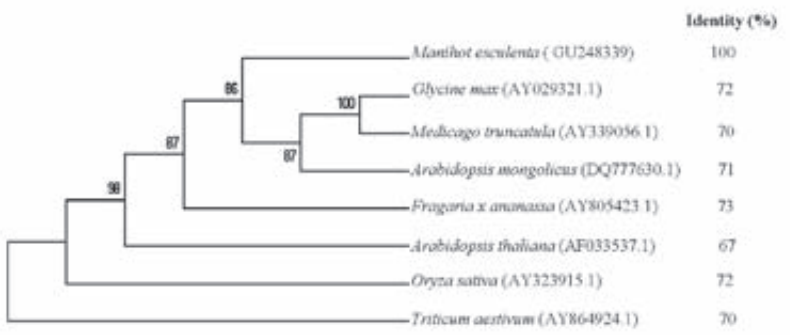

Figure 2 Phylogenetic analysis of aligned amino acid sequences deduced from *MeZIP* conserve cDNA and other *ZIP* genes. For sources, numbers in parentheses denote GenBank accession numbers. To estimate phylogenetic relationships from the deduced amino acid sequences, a bootstrap was generated after 1,000 cycles of bootstrap replicates, using the neighbor-joining method of the MEGA 4 software program.

Table 1 Relative fold change in MeZIP expression in young leaves, stem and fibrous roots of three cassava varieties, namely HB80, KU50 and R1 (A). Cassava tissue samples were grown at different levels of $ZnSO_4.7H_2O$ (0, 0.037, 0.075, 0.15 and 0.22 mM in MS media (B), and harvested at 4, 5, 6, 7 and 8 week after subculture (C).

		Young leaf	Stems	Fibrous roots
	HB 80	4.6939	2.4014	1.1336
A	KU 50	3.8775	4.4521	1.4314
	R 1	4.7382	4.7751	1.8801
	0 mM	6.2052	5.7594	3.4336
	0.037 mM	1.5703	2.1082	0.5034
B	0.075 mM	1.0000	1.0000	1.0000
	0.15 mM	0.0231	2.8571	2.1988
	0.22 mM	0.3804	1.6403	0.2716
	4 week	4.1812	2.8524	1.2339
	5 week	5.7494	6.5162	2.4903
C	6 week	4.5974	5.2284	1.7348
	7 week	3.8132	2.6301	1.3697
	8 week	3.8384	2.1564	0.5797

coordination between gene expression, presumed to lead to the synthesis of more transporters and increased uptake, and the availability of zinc in the medium. During growth development, the expression pattern of *MeZIP* gene in young leaves, stems and fibrous roots showed that the maximal transcript levels of *MeZIP* could be observed at 5-6 week, after which the level gradually decreased (Table 1C).

Response of cassava plants to Zn

The response of cassava to different Zn levels of supply was studied. Zinc concentrations in MS media, to which the plants were exposed, were increased to 0, 0.037, 0.075, 0.15 and 0.22 mM $ZnSO_4.7H_2O$, respectively, to see if plants showed any visible phenotype. Overall plant size was smaller when the plants were grown under Zn-deficient and high-Zn conditions, compared to the sufficient-Zn media (Figure 3). The fresh mass of young leaves, stems and fibrous roots was reduced 13-, 5- and 30-fold, respectively, under Zn-deficient conditions (0 mM) and reduced twofold under high-Zn conditions(0.22 mM) compared to 0.15 mM Zn (Figure 4; A-1, B-1, C-1). The reduction in the dry mass of young leaves, stems and fibrous roots was 9-, 3- and 20-fold, respectively, under Zn-deficient conditions (0 mM) and twofold under high-Zn conditions (0.22 mM), compared to 0.15 mM Zn (Figure 4; A-2, B-2, C-2). Furthermore, the leaves were smaller in plants grown with either low or high levels of Zn (Figure 3).

Determination of *MeZIP* gene copy number

Genomic DNA was extracted from young leaf tissue of Thai cassava. Southern analysis was used to determine the *MeZIP* gene in the cassava genome. The isolated genomic DNA migrated as a single band with a size of approximately 10 kb. *Sac*I, *Sal*I and *Pst*I endonucleases were used individually to completely digest the cassava genomic DNA. Using the *MeZIP* probe, a single band of approximately 10 kb was observed using digested *Pst*I. The *Sac*I lane showed two bands of 8.0 and

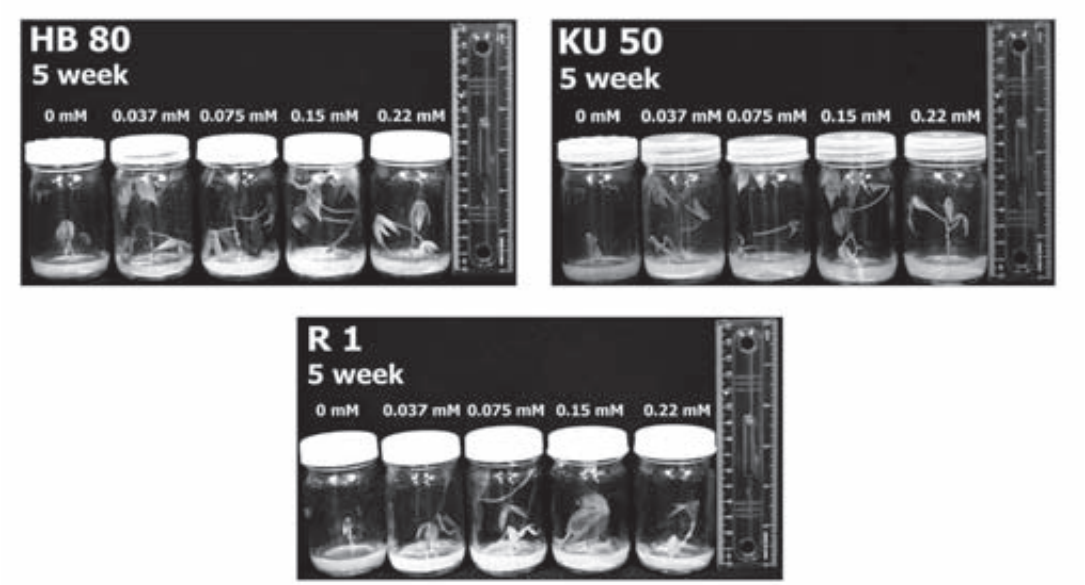

Figure 3 Phenotypic differences of cassava plants (cv. HB80, KU50 and R1) in response to deficient-, sufficient- and high- levels of Zn. Cassava plants grown on tissue media under conditions of Zn-deficiency (0 and 0.037 mM Zn), Zn-sufficiency (0.075 and 0.15 mM Zn) and high Zn (0.22 mM Zn) after 5 week.

6.5 kb, whereas two bands of 9.0 and 0.9 kb were detected in the *Sal*I lane (Figure 5).

DISCUSSION

Zinc is an essential micronutrient for cassava growth, but relatively few studies have examined the mechanisms and regulation of Zn uptake. In the current study, the zinc transporter gene was cloned and characterized for cassava. Partial sequences of cDNA corresponding to the *ZIP* gene were obtained by RT-PCR of the total RNA isolated from fibrous roots.

The differential expression of the *ZIP* gene was observed in several parts of cassava. Among three parts of the plant (young leaves, stems and fibrous roots), *MeZIP* expression was high in young leaves. This result was in contrast to that of a study using *Arabidopsis* (Grotz *et al.*, 1998), where the highest levels of *ZIP1* and *ZIP3* genes were found in the roots and *ZIP4* was high in the shoots and roots. The differential expression of *MeZIP* in all parts of the plant correlated with the physiology of cassava growth, in which the fresh and dry mass of young leaves, stems and fibrous roots were used to support the vegetative growth of the cassava. The results suggested that *MeZIP* might be involved in zinc uptake by cassava at different developmental stages.

The Zn-deficiency phenotypes in cassava plants were studied. In general, plants showed all the characteristics that have been described previously for Zn-deficient plants in several other species: reduction of internodal growth with an enhanced rosette-like development; small and discolored leaves; poor root formation; and reduced seed and fruit production (Bergmann, 1992; Marschner, 1995 and Broadley *et al.*, 2007). The reduced height of Zn-deficient (0 mM Zn) plants compared with Zn-sufficient (0.15 mM Zn) plants could have been due to a decrease in the level of the growth hormone, auxin, that correlated

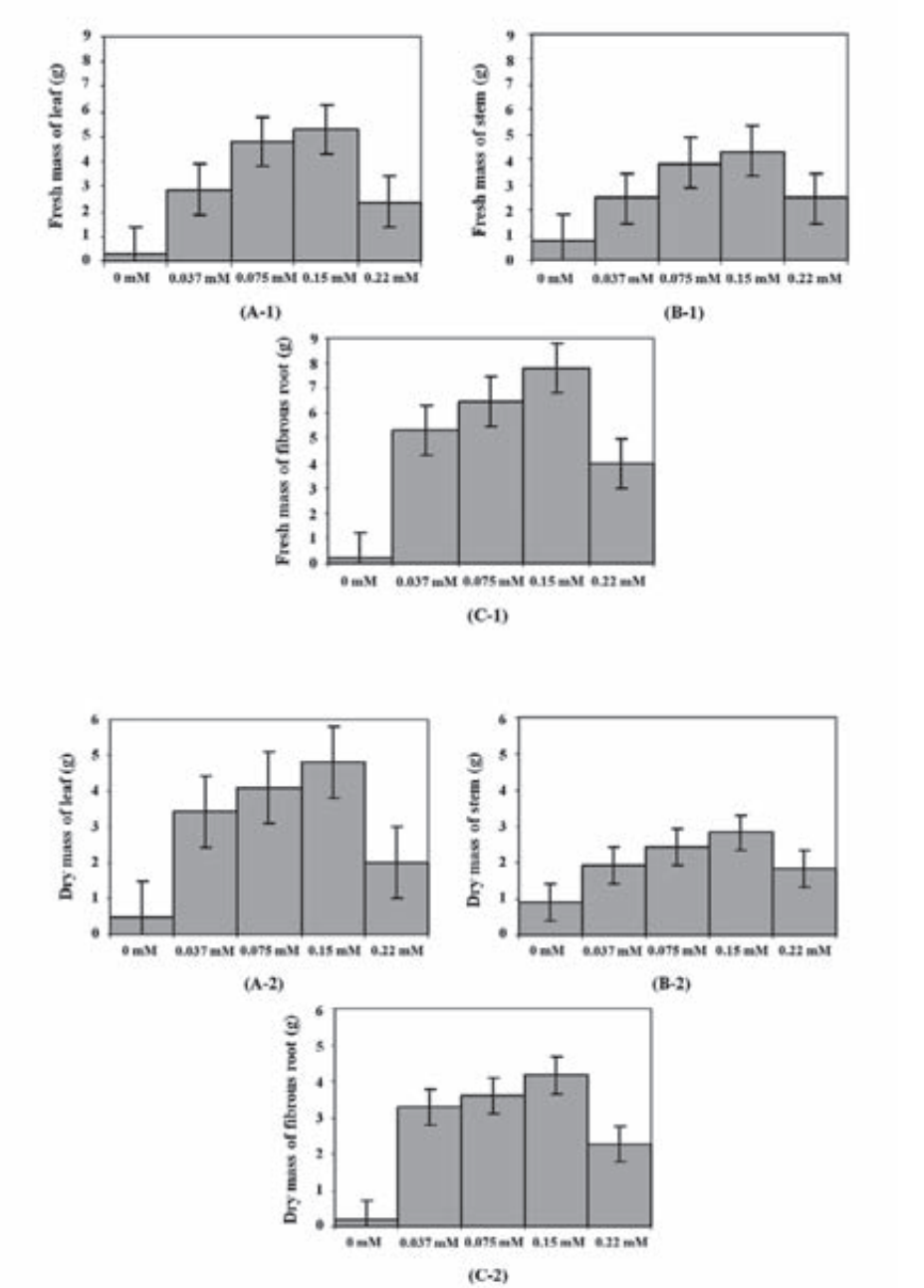

Figure 4 Fresh and dry mass of young leaves, stems and fibrous roots of cassava grown under conditions of sufficient Zn (0.075, 0.15 mM Zn); high Zn (0.22 mM Zn) and deficient Zn (0, 0.037 mM Zn). Fresh mass of young leaves (A-1), stems (B-1) and fibrous roots (C-1); dry mass of young leaves (A-2), stems (B-2) and fibrous roots (C-2).

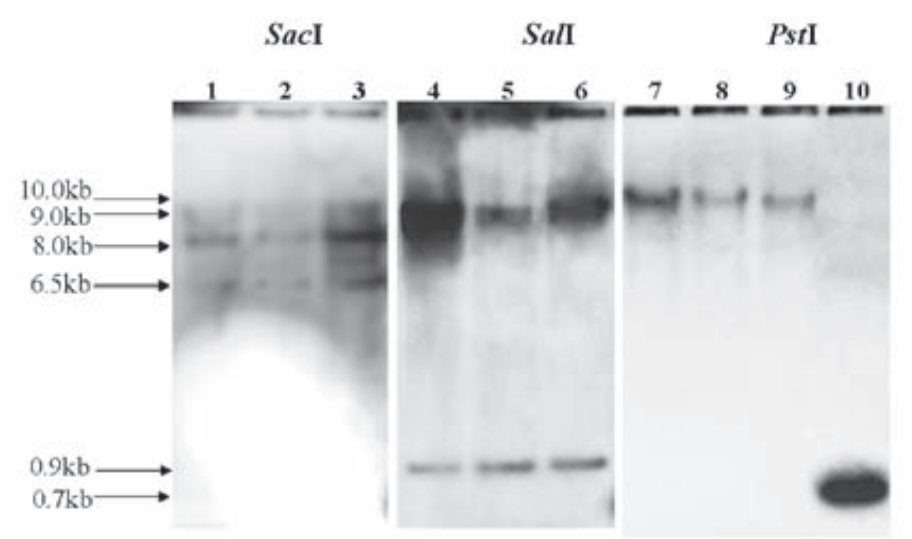

Figure 5 Southern hybridization of cassava genomic DNA, using the *MeZIP* probe: lanes 1, 2, 3 = cassava DNA (HB80, KU50 and R1, respectively) digested with *Sac*I; lanes 4, 5, 6 = cassava DNA (HB80, KU50 and R1, respectively) digested with *Sal*I; lanes 7, 8, 9 = cassava DNA (HB80, KU50 and R1, respectively) digested with *Pst*I. Lane 10 = plasmid control digested with *Eco*RI.

with decreased Zn levels in the plants. A previous study on the relationship of Zn and auxin has shown that a decrease in auxin precedes the appearance of visible symptoms of Zn deficiency in tomatoes and sunflowers, suggesting that Zn is required for auxin production. The effect of a reduction in auxin levels was only observed in Zn-deficient plants and not in Mn-deficient and Cu-deficient plants (Skoog, 1940). The 'little leaf' is typical of Zn-deficiency, as it is also found in other plants. The increasing chlorosis in the leaf is assumed to be due to the effect of a decrease in Zn in the photosynthetic enzymes. Zn deficiency in plants is also known to result in an impaired response to oxidative stress, which is likely due to a reduction in superoxide dismutase levels (Hacisalihoglu *et al*., 2003). Sharma *et al*. (1995) showed a requirement of Zn for stomatal opening in cauliflowers. Zn-deficiency induced: increases in epicuticular wax deposits, lamina thickness, a degree of succulence, a water saturation deficit, diffusive resistance, and proline accumulation; and decreases in carbonic anhydrase activity, water potential, stomatal aperture and transpiration in the leaves of cauliflower plants (Sharma *et al*., 1995).

Southern blot analysis was carried out, using the partial sequence of *ZIP* as a probe. When the Southern blot was probed with *ZIP*, the presence was shown of a single band in the *Pst*I-treated samples and of two bands in the *Sac*I and *Sal*I samples. According to the restriction map of *ZIP*, every endonuclease used in this experiment was cut only once at one specific site, which suggested that there is more than one copy of *MeZIP* in the cassava genome. Correlating with the cloning experiment, *ZIP* was more abundant and was easily detected in the cassava genome. Different patterns appeared after digestion that indicated different gene encoded zinc transporters.

CONCLUSIONS

A conserved cDNA sequence of the *MeZIP* gene was identified and the expression was characterized in young leaves, stems and fibrous roots. According to RT-PCR analysis and phenotypic characteristics, *MeZIP* might play an important role in the zinc uptake process for growth development, especially through the expression of *MeZIP* in fibrous root organs. Further investigation of *MeZIP* expression in different stages of root development and vegetative growth will clarify its roles. The copy number determination of the *MeZIP* gene indicated that *MeZIP* exists in the cassava genome as a multi-copied gene. The information obtained from this experiment including sequence, sequence analysis and, mostly importantly, gene expression, confirmed the complexity of the zinc uptake process in cassava and suggested that further investigation is needed.

ACKNOWLEDGEMENTS

This research was supported by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office (PERDO), Commission on Higher Education, Ministry of Education, and by the Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok.

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