Functional characterisation of the barley ZIP7 zinc transporter

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Abstract

Zinc (Zn) is an essential micronutrient for the function of many biological processes. Zn deficiency therefore affects normal growth and development in all living organisms. Many cropping soils are low in Zn, leading to widespread Zn deficiency in cereal crops. This reduces crop yield and grain nutrition value, which increases the risk of Zn deficiency-related health problems, especially for women and children in developing countries. Increasing Zn density in cereal grains (biofortification) is a potentially effective strategy to alleviate widespread Zn malnutrition in humans. However, grain Zn biofortification is restricted by the translocation of Zn from roots to shoots and from shoots to grains. So far, little is known about the metal transporters involved in these two limiting processes. Identification and characterisation of these transporters are crucial for Zn biofortification. The Zn regulated, Iron regulated-like Protein (ZIP) family is a group of transporters controlling cellular Zn influx, and could be important for Zn uptake and translocation in plants. In this study, barley (*Hordeum vulgare* L.) was used as a model plant to identify and characterise new ZIP transporters. Nine new HvZIP members were identified, and transcript analyses of 13 *HvZIP* genes revealed that eight of them were induced in Zn-deficient plants. *HvZIP7* was further characterised as there is no available functional information for this transporter. *HvZIP7* is primarily expressed in vascular tissues of roots and shoots and localises in the plasma membrane when expressed in onion epidermal cells. The over-expression of *HvZIP7* resulted in a specific increase of Zn translocation from roots to shoots and Zn accumulation in shoots and grains when Zn is abundant in the growth media, suggesting that HvZIP7 mediates Zn translocation and/or retranslocation. The enhanced Zn accumulation in plants did not affect plant growth or grain yield. Furthermore, the over-expression of *HvZIP7* did not enhance Cd accumulation in shoots, which differs from the over-expression of heavy metal ATPases. In addition, transgenic *HvZIP7* plants could achieve 30% higher Zn concentration in grains than

that of controls when grown with a low dose of Zn fertilisers under conditions similar to the field, indicating that our findings would have direct applications to Zn biofortification and the improvement of plant Zn nutrition in cereals.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Jingwen Tiong and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Jingwen Tiong Date

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Glossary of abbreviations

Abbreviation	Full term
β	Beta
°C	Degree Celsius
µg	Microgram(s)
µM	Micromolar
µmol	Micromole
ATP	Adenosine triphosphate
At	*Arabidopsis thaliana*
BAC	Bacterial artificial chromosome
BLAST	Basic Local Alignment Search Tool
Bd	*Brachypodium distachyon*
C-terminal	Carboxyl terminal
CA	Carbonic anhydrase
CaMV	Cauliflower mosaic virus
cDNA	Complementary deoxyribonucleic acid
CDF	Cation diffusion facilitator
CoT	Cobalt transporter
cv.	Cultivar
DNA	Deoxyribonucleic acid
DTPA	Diethylenetriaminepentaacetic acid
dUTP	Deoxyuridine triphosphate
DM	Dry matter
DW	Dry weight
ELISA	Enzyme-linked immunosorbent assay
FAA	Formalin-acetic acid-alcohol
FAOSTAT	Food and Agriculture Organisation Statistical Database
g	Gram(s)
GFP	Green fluorescent protein
Gm	*Glycine max*
GUS	Beta-glucuronidases
HEDTA	Hydroxyethyl ethylenediamine triacetic acid
HMA	Heavy metal ATPase
Hr	Hour(s)

Hv	*Hordeum vulgare*
IAR	Indole-3-acetic acid resistant
IBSC	International Barley Genome Sequencing Consortium
ICP-OES	Inductively coupled plasma optical emission spectrometry
IRT	Iron-regulated transporter
kg	Kilogram(s)
Le	*Lycopersicon esculentum*
LSD	Least significant difference
M	Molar
m	Meter(s)
MEGA	Molecular Evolutionary Genetics Analysis
mg	Milligram(s)
min	Minute(s)
MSU	Michigan State University
Mt	*Medicago truncatula*
MTP	Metal tolerance protein
N-terminal	Amino terminal
NADPH	Nicotinamide adenine dinucleotide phosphate
NAS	Nicotianamine synthase
NCBI	National Center for Biotechnology
OPT	Oligopeptide transporter
Os	*Oryza sativa*
OX	Over-expressing
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PM	Plasma membrane
PT	Phosphate transporter
Pv	*Phaseolus vulgaris*
RGAP	Rice Genome Annotation Project
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcript polymerase chain reaction
s	Second(s)
SE	Standard error
SOD	Superoxide dismutase

ssp.	Sub-species
Ta	*Triticum aestivum*
TAIR	The Arabidopsis Information Resource
Tc	*Thlaspi caerulescens*
Td	*Triticum turgidum*
Tg	*Thlaspi goesingense*
Tj	*Thalspi japonicum*
TM	Trans-membrane
Tr	Transgenic
Trx	Thioredoxin
U	Unit(s)
WT	Wildtype
YS	Yellow strip
YSL	Yellow stripe-like
ZIP	Zinc-regulated, iron-regulated transporter-like protein
ZnT	Zinc transporter
ZRC	Zinc resistant conferring
ZRT	Zinc regulated transporter
ZTP	Zinc transporter protein