Transport and Deposition of Zinc in the Developing Barley Grain

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INTRODUCTION

The primary aim of this project was to study the genetic and molecular mechanisms underlying Zn transport and deposition in the cereal grain. Barley was used as a model plant because post-genomic tools are available for it.

In small-grained cereals, Zn and Fe are primarily stored together with phytate in protein storage vacuoles in the aleurone layer and the embryo. The deposition pattern of Zn and Fe has consequences for human mineral nutrition. During milling, outer tissues and the embryo are removed from the grain, which results in a virtual depletion of Zn and Fe.

Zinc and Fe deficiencies are the most widespread micronutrient deficiencies in humans. These deficiencies are particularly common in developing countries, where diets are rich in cereal-based foods, which serve as excellent suppliers of carbohydrates but have low concentrations of bioavailable Zn and Fe. Some of the major health consequences of these deficiencies include retardation of growth, anaemia and impaired immune function. This can be explained by the facts that about 3% of all genes in humans encode proteins with Zn-binding motifs and that Zn is required as a co-factor in more than 300 enzymes.

Elucidation of the mechanisms responsible for transport and deposition of Zn in the barley grain was approached by performing transcript profiling of different tissues of the grain. A barley Zn-related transcriptome microarray was developed which comprised the majority of genes known to be involved in Zn transport, deposition and homeostasis. Relevant genes were identified by searching barley Expressed-Sequence Tag (EST) databases for orthologs to all identified and annotated Zn transporters, chelators etc. in *Arabidopsis* and rice. The Zn-transcriptome array contained unique oligonucleotides representing 129 different ESTs from 13 different gene families.

The array was used for expression studies of the Zn transcriptome in tissue sections of barley grains at different developmental stages of plants subjected to high and low Zn regimes. Three populations of the barley cultivar ‘Golden Promise’ were grown in a stone wool substrate to precisely control the supply and composition of nutrients to the plants. The plants were supplied with nutrient solutions containing 0, 3μM and 300μM Zn, respectively. Seeds from these plants were collected 14, 23 and 30 days after pollination, before and after the foliar application of 5 mM $ZnSO_4$.

The laser capture microdissection technique was subsequently used to isolate transfer cells from the unloading region, aleurone cells and cells from the endosperm. Ribonucleic acid (RNA) was extracted from isolated cells, amplified and used for microarray hybridizations and real-time polymerase chain reaction (RT-PCR) analyses to establish the temporal and spatial expression patterns of relevant genes.

METHODS

Bioinformatics

In silico analyses were performed by searching available barley EST databases for sequence information of genes from *Arabidopsis* and rice known to be involved in transport and deposition of Zn. Unique oligonucleotides (50–70 mers) were designed to represent each EST.

Microarray

The amount of 20 μM of each oligonucleotide was spotted on a Nexterion A+ glass slides in 50% DMSO.

Laser Capture Microdissection (PALM System)

Tissue sections from paraffin embedded seeds were prepared. Cells from different tissues of the grain were subsequently isolated and captured in a buffer containing RNase inhibitor and Proteinase K.

RNA Extraction

RNA was extracted from the samples using the PicoPure™ RNA isolation Kit (Arcturus).

RNA Amplification

The RNA was amplified using the RiboAmp™ RNA Amplification Kit (Arcturus). The resulting aRNA was subsequently labelled with Cy3 or Cy5 using a direct incorporation method.

RESULTS AND DISCUSSION

Three barley populations were supplied with nutrient solutions containing 0, 3 or 300 μM Zn to induce responses to Zn deficiency and Zn excess. Element analyses of leaves were performed for all populations to determine their Fe and Zn status (Table 1).

Table 1. Zinc and Fe content of barley leaves after exposure to different Zn concentrations.

	Leaf content	
Zn supply	Zn	Fe
(μM)	(mg kg^{-1})	(mg kg^{-1})
0	24	164
3	43	103
300	819	71

The Zn content of plants grown without Zn may have come from nutrient reserves of the embryo. The Fe concentration of the nutrient solutions was constant for all populations. Thus, the high Fe content of the Zero-Zn population might be an indication of Zn-deficiency related stress in plants, since the up-regulation of Zn transporters in Zn-deficient plants will result in increased uptake of other metal ions, including Fe.

Preliminary analyses of RNA from isolated cells revealed that <100 cells (50 – 100 pg) can be amplified to yield ~7 μg cDNA, which is sufficient for microarray hybridizations.

REFERENCES

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