Expression Analysis of Metal-Related Genes in Flag Leaves of Diverse Rice Germplasm

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INTRODUCTION

Rice, one of the most important food crops, is a poor source of essential nutrients such as Zn and Fe. Much of the world's micronutrient malnutrition is found in developing countries where rice is the staple food. Therefore, engineering rice plants with enhanced seed nutrients concentrations will help to minimize current problems of human micronutrient deficiencies. To assist molecular breeding or transgenic efforts, more information is needed about the molecular players that help mobilize nutrients into and throughout the plant, especially to the developing seeds. In this study, we focused on metal-related gene expression in the flag leaf because this leaf is the major source of phloem-delivered photoassimilates and nutrients for seeds. Based on an earlier expression study of 36 metal-related genes in rice (Narayanan et al. 2007), we selected eight of the more highly expressed genes for further study. Several diverse rice genotypes that exhibit different seed concentrations of Zn and Fe were chosen for expression analysis. This allowed us to compare genotypic differences in gene expression with differences in seed nutrient concentrations.

METHODS

Flag leaves and immature grains were collected from the field at the USDA-ARS Dale Bumpers National Rice Research Center in Stuttgart, AR, USA. Flag leaves were immediately frozen in liquid nitrogen in the field and subsequently were shipped frozen to the Houston, TX lab. Grains were separated from hulls and dried in a 70˚C oven. Tissue samples were collected from 14 accessions; all were part of the USDA Rice Germplasm Core Collection (PI 346827, PI 393180, PI 400275, PI 389874, PI 584587, PI 373122, CIor 12285, PI 231648, PI 215485, PI 291542, PI 340889, PI 439628, PI 223518, and PI 162365). Total ribonucleic acid (RNA) was extracted from three flag leaves of each accession. Gene expression was analyzed for the following genes (Genbank numbers in parentheses): *OsIRT1* (AB070226), *OsZIP1* (AY302058), *OsYS17* (AB190917), *OsYS18* (AB190918), *OsYSL18* (AB190926), *OsNRAMP2* (L81152), *OsNRAMP7* (AK071485), and *OsNAS1* (AB046401) using quantitative polymerase chain reaction (PCR) techniques as previously described (Narayanan et al. 2007). Delta C_T values were calculated relative to the expression of 18S transcripts. For immature grains, nutrient concentrations were analyzed on acid-digested samples using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

RESULTS AND DISCUSSION

Quantitative PCR

Transcripts of eight putative metal-related genes (*OsIRT1, OsZIP1, OsYS17, OsYS18, OsYSL18, OsNRAMP2, OsNRAMP7* and *OsNAS1*) were detected in all 14 accessions tested (Fig. 1). For most of the genes, there were no or only minor differences in gene expression between the accessions. The notable exception to this was *OsZIP1*, which was quite variable across the accessions. *OsZIP1* also tended to be expressed at lower levels than the other genes (Fig.1; note that a higher ΔC_T value indicates a lower level of expression).

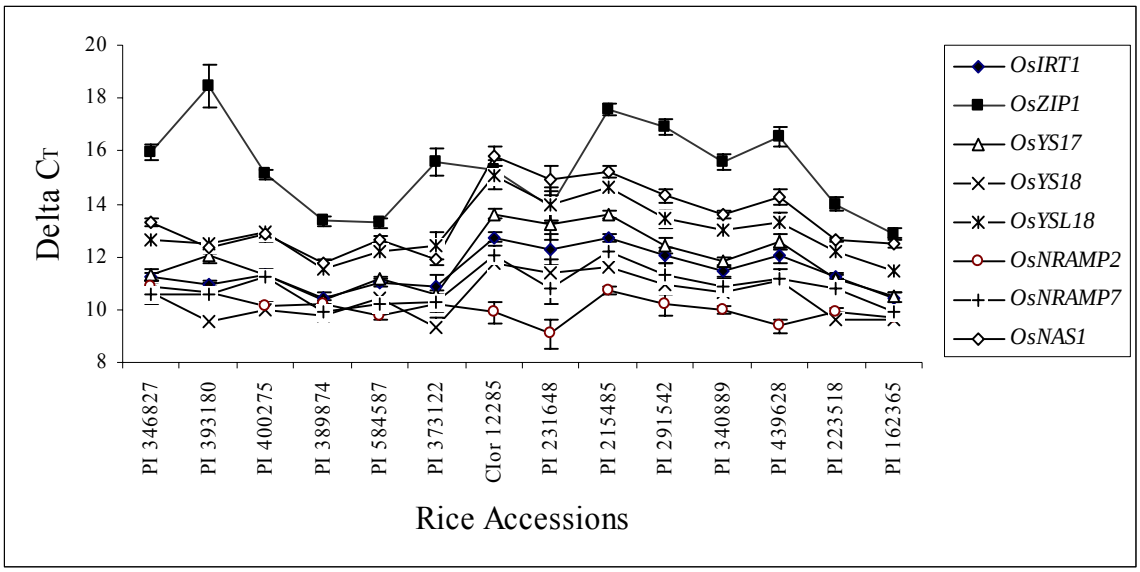

Fig.1. Expression profiling of 8 putative metal-related genes in flag leaves of different rice accessions

Elemental Analysis

The elemental analysis of immature grains showed broad variation in seed nutrient concentrations amongst the accessions tested (data not shown). Preliminary analysis revealed significant correlations between seed Zn concentration and the flag leaf expression of certain genes (data not shown).

CONCLUSIONS

Eight metal-related genes were found to be expressed in flag leaves of all 14 accessions, suggesting that the products of these genes contribute to the movement of metals between and within various cellular/subcellular leaf compartments, at least during the stage of grain fill in rice. Correlations between seed Zn concentration and the expression of certain genes implies that a mechanistic link between flag leaf processes and seed Zn delivery can be deciphered, and may lead to new strategies for enhancing the concentration of Zn in seeds.

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