Title
Characterization of a mitochondrial iron regulated gene (MIR) in rice

Permalink
https://escholarship.org/uc/item/17g4b57m

Authors
Bashir, Khurram
Ishimaru, Yasuhiro
Fujimoto, Masaru
et al.

Publication Date
2009-08-07

Peer reviewed
Introduction

Iron (Fe) is an essential micronutrient for plants. In plants, Fe is essential for several cellular processes such as respiration, chlorophyll biosynthesis, and photosynthetic electron transport. Limited Fe content impairs the metabolic and respiratory activities of the mitochondria, whereas excess Fe can be toxic owing to the generation of oxidative stress through the formation of radicals. The proteins involved in mitochondrial Fe homeostasis have not been characterized in plants. Here, we report the cloning and characterization of a mitochondrial iron-regulated protein (MIR) from rice. The data suggest that MIR is a rice-specific gene that plays a significant role in Fe homeostasis via mitochondria.

Materials and Methods

The MIR T-DNA knockout rice line (mir) was obtained from the rice functional genomics database maintained at http://signal.salk.edu/cgi-bin/RiceGE. Mutant plants were isolated by PCR-based screening using the T-DNA right border-specific primers 5′-GTTACGTCCTGTAGAAACCCCAACCC-3′ and 5′-ATACGCTGGCCTGCCCAACCTTTTCG-3′. Two MIR internal primers (5′-CGTCATGGTCTTCGGTCTCCTACGTGCTCG-3′ and 5′-GCTAGTCGTTGTCACACAGTCAACAAAGA-3′) located outside the T-DNA integration site were used to confirm the T-DNA integration site and homozygous status of mir. Rice seeds were germinated on wet filter paper and grown under Fe-sufficient and Fe-deficient conditions. Microarray analyses and elemental analyses of WT and mir knockout plants by inductively coupled plasma atomic emission spectrometry (SPS1200VR; Seiko, Japan) were performed as described previously (Ishimaru et al., 2007).

The full-length ORF of MIR was subcloned into pH7WGF2 (Karimi et al., 2002) using the LR recombination reaction (Invitrogen, Japan). BY-2 cells were transformed and examined as described previously (Li et al., 2000).

Results and Discussion

MIR was identifiable through an oligo microarray analysis, because its expression was greatly up-regulated in response to Fe deficiency in roots as well as shoots. MIR is located on rice chromosome 12 and is composed of three exons. When transiently expressed in tobacco BY-2 cells, MIR-sGFP localized to mitochondria. When grown hydroponically in the presence or absence of Fe, the growth of mir plants was significantly impaired compared with WT plant growth. The shoot lengths in mir plants were 14% and 18% less, and the root lengths were 24% and 43% less than the respective lengths in WT plants, when cultured in the presence and absence of Fe, respectively. The mir plants accumulated higher Fe concentrations than WT...
plants grown under the same conditions. Root Fe concentrations were significantly higher in \textit{mir} plants, and shoot Fe concentrations in \textit{mir} plants were 2.3 and 2.2 times those in WT plants under Fe-sufficient and Fe-deficient conditions, respectively. Furthermore, \textit{mir} plants grown under Fe-deficient conditions accumulated as much Fe as did WT plants grown under Fe-sufficient conditions, yet showed symptoms of Fe deficiency. These results indicate that although \textit{mir} plants had enough Fe in shoot tissues, the Fe was probably not available for physiological functions, resulting in symptoms typical of Fe-deficiency stress.

The 44K microarray analysis revealed that \textit{mir} roots grown under Fe-sufficient conditions had significantly increased expression of genes normally up-regulated in response to Fe deficiency, including \textit{OsYSL2}, \textit{OsYSL15}, \textit{OsIRT1}, \textit{OsNAS1}, \textit{OsNAS2}, \textit{OsNAAT1}, and \textit{OsDMAS1}, compared with expression in WT plants. The up-regulation of these genes appears to be responsible for the high accumulation of Fe in \textit{mir} plants and at the same time indicates that this Fe may not be available for physiological functions, given that plant Fe deficiency-inducible genes are only up-regulated under Fe-deficient conditions in WT plants. No homologs of MIR were found in any organism, indicating that \textit{MIR} is a recently evolved rice-specific gene. As MIR is not homologous to any known protein and does not contain any conserved domains, it is difficult to predict its function. Nevertheless, its importance in the Fe-deficiency response cannot be overlooked, as \textit{MIR} is clearly induced by Fe-deficiency stress and Fe homeostasis is significantly impaired in the \textit{mir} mutant. Based on its localization to mitochondria, MIR appears to play a role in mitochondrial Fe homeostasis.

Acknowledgement

We are thankful to Dr Yoshiaki Nagamura of the Rice Genome Project and the NIAS DNA Bank (Tsukuba, Japan) for support with the microarray analysis. This work is supported by a grant from Ministry of Agriculture, Forestry and Fisheries of Japan (Green Technology Project IP-5003).

Reference: