Introduction

Fe is an essential micronutrient for plants significantly effecting plant growth and development. Mitochondrion, the power house of cell, requires Fe for various cellular functions including heme synthesis and synthesis of various Fe-S proteins. Thus, regulating the mitochondrial Fe homeostasis is extremely important for plants. MRS3 and MRS4 are yeast (*Saccharomyces cerevisiae*) mitochondrial substrate carrier family proteins transporting Fe into mitochondria under conditions of low Fe availability. MRS3-MRS4 knockout yeast (*mrs3mrs4*) accumulates more Fe compared to WT strain and unable to grow well when grown under Fe-deficient conditions. The high accumulation of Fe is a result of increased expression of yeast vacuolar Fe transporter Ccc1p transporting Fe to vacuole, rendering the cytoplasm Fe deficient. As a result, the Fe uptake system is triggered ultimately accumulating more Fe (Li and Kaplan, 2004). On the other hand, the mechanisms of mitochondrial Fe homeostasis are not well understood in plants. Here we report the cloning and characterization of a rice mitochondrial Fe transporter (OsMIT). The characterization of OsMIT will be helpful to understand the mitochondrial Fe homeostasis and to develop strategies to mitigate the Fe deficiency problem.

Materials and Methods:

OsMIT was identified by screening rice T-DNA knockout mutants for Fe deficiency phenotypes. The full length ORF of *OsMIT* was amplified with 5”-CACCATGGCCGCCGACTACCGCACC-3” and 5”-TTATTTCCTTCTTTTCTCGTTGA-3” as forward and reverse primers, respectively, cloned in to pENTR TOPO (Invitrogen) and subcloned into pHY23 using LR reaction (Invitrogen, Japan). Yeast strains *mrs3mrs4* defective in mitochondrial Fe transport under low Fe availability were used to check the function of OsMIT. To check the subcellular localization of OsMIT, the full length *OsMIT* was subcloned in to pH7WGF2 (**Karimi et al., 2002**) using LR recombination reaction (Invitrogen, Japan). Tobacco BY-2 cells were transformed and the expression of GFP was observed as described (**Li et al., 2000**).

Results and discussion:

OsMIT was identified by T-DNA knockout mutant screening as a member of mitochondrial substrate carrier family and a homolog of yeast mitochondrial Fe transporters MRS3 and MRS4. This mutation was severely chlorotic and the growth was significantly impaired. *OsMIT* is located on rice chromosome 3, ORF consists of 987 nucleotides and encodes a predicted polypeptide of 329 amino acids. The 44 K microarray analysis indicated that the expression of *OsMIT* is not up-regulated under Fe

deficiency treatment. When transiently expressed in tobacco BY-2 cells, OsMIT-sGFP localized to mitochondria, confirming that OsMIT is a mitochondrial protein. *OsMIT* was expressed in yeast mutant *mrs3mrs4* defective in mitochondrial Fe uptake under conditions of low Fe availability. The expression of *OsMIT* complemented the growth defect of *mrs3mrs4* mutant. Moreover, it also reversed the high Fe accumulating phenotype of *mrs3mrs4*. These results confirmed that OsMIT have a role in mitochondrial Fe homeostasis. The cloning and characterization of *OsMIT* is a significant advance in understanding the mitochondrial Fe homeostasis.

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Reference

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