

Heavy Metal ATPase 3 (HMA3)

and Its Roles in Cadmium Sequestration in Rice Cell Vacuoles

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Introduction

According to the World Health Organization, cadmium (Cd) is among the top 10 most toxic chemicals for public health (WHO, 2007). Cadmium occurs naturally in the soil at low nontoxic quantities. In the earth crust, its concentration is generally between 0.10 mg/kg DW and 0.15 mg/kg DW (Dry Weight) with much higher levels in sedimentary rocks which is approximately 10 mg/kg DW. However these concentration increases to harmful quantities through activities such as mining and metal processing operations, burning fuels, making and using phosphate fertilizers, and disposing of metal products (IDPH, 2015).

Through crops such as rice, cadmium enters into the human bodies, causing renal and lung cancer, kidney failure and bone lesions. Rice is a staple food which is one of nature's great scavengers of metallic compounds especially cadmium. Toxic metals such as cadmium and arsenic are transported in the plants via the same transporters as those of micronutrients such as zinc (Zn). One of the mechanisms that aid in detoxification is subcellular compartmentalization into the vacuoles. Heavy metal ATPase3 (HMA3) is a vacuolar membrane transporter that is known to aid in subcellular compartmentalization of Cd in the vacuoles (Figure 1).

The main goal of this research is to assess the relative expression of HMA3 gene in two different rice lines which differ in their accumulation of Cd in the grains. This will give us an idea about the role of HMA3 in subcellular sequestration of Cd in rice.

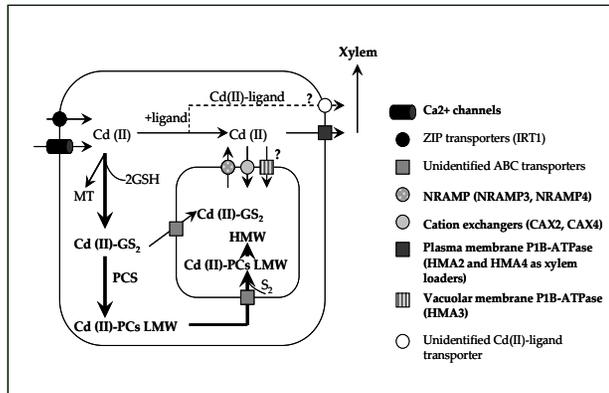


Figure 1: Proposed mechanisms for root intracellular Cd transport (Adapted from Verbruggen et al., 2009).

Materials & Methods

50 seeds of Rice (*Oryza sativa*) Line, 310546 – *Oryza Sativa Indica* and 310428 – *Oryza Sativa Japonica* were surface sterilized. The seeds were then initially germinated in between two moist filter papers in a petri dish and kept in a dark space for 4 days. After, they were transferred and germinated in moist metro mix 360 soil and kept in the greenhouse at the New Science Hall, Lehman College. A week after, germinated seedlings were transferred to pots with roots dipped into modified Johnson's nutrient. The nutrient solution comprised of 250 mM CaCl₂, 5.0 mM MnSO₄, 250 mM H₃BO₃, 20.0 mM ZnSO₄, 5.0 mM CuSO₄, 5.0 mM H₂MoO₄, 1.0 mM NiCl₂, 20 μM FeHEDTA. N(2Hydroxyethyl)ethylenediaminetriacetic acid, and the solutions were buffered with 2 mM MES[2(morpholino)ethanesulfonic acid] so that the pH level can be regulated at 5.5. The solution setup were aerated and was replaced weekly.

In the experimental design for these two lines, there was a vegetative control group and a vegetative treated group. The treated group had 0.5 μM CdSO₄ in addition to the nutrient solution. Chlorophyll measurements were recorded every other day using a SPAD chlorophyll meter. After 4 weeks, plants were harvested and cut 3 cm above the root for an hour of xylem extraction. Leaves, stems and root were separated from each other and frozen in liquid nitrogen for RNA extraction according to the QIAGEN kit handbook. Using a Nano Drop ND-1000 Spectrometer, a quality control checkup was performed on the extracted RNA sample so as to determine the quantity of RNA that has been isolated, its purity and its integrity. Extracted RNA sample was DNase treated in order to guard against any DNA contamination. In order to obtain a secured treated RNA, the DNase treated sample was incubated at 37°C for 30 min then 1 μl Stop Solution 20mM ethylene glycol tetra acetic acid (pH 8.0) was added and incubated once again at 60°C for 10 min using a thermal cycler.

To obtain cDNA which acts as a template for gene expression, a reaction known as Reverse transcription (RT-PCR) was performed by adding certain enzymes and 1 μl of Reverse Transcription Random Primers, then incubating it at different temperatures at specific time. Using primers ordered from life technologies for the targeted genes (HMA3 and Actin), real time PCR was performed, the targeted gene were amplified and fluorescence intensity of the targeted genes obtained which is converted to amplification curve. This procedure would help determine the quantity of the targeted genes that were expressed/replicated in the leave tissue, root tissue and xylem sap which is the bases for accomplishing the objective of this research.



Fig 2 *Oryza sativa* grown in metromix



Fig 3 *Oryza sativa* grown in hydroponics



Fig 4 Relative Chlorophyll content measurement



Fig 5 Thermal Cycler

Results & Conclusion

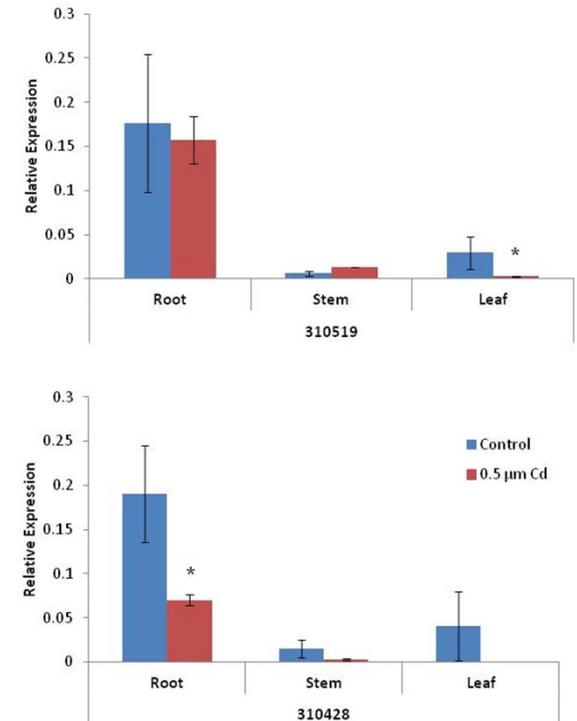


Figure 6: Relative expression of HMA3 in 310519 and 310428 rice lines during the vegetative stage. Values represent mean ± s.e

- There is no difference in relative expression of HMA3 in both high and low Cd accumulator lines in most tissues in both lines during the vegetative growth.
- This indicates that HMA3 does not have a significant role in subcellular sequestration of Cd in both these lines.
- Future studies will be conducted at anthesis to assess the role of HMA3 in Cd detoxification in these lines.

References

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