Bronx STEM Scholars Program

Glutathione, Phytochelatin, and HMA3 and Their Roles in Cadmium Chelation and Detoxification in Rice

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Introduction

Cadmium (Cd) is a toxic, non-essential heavy metal and environmental pollutant (Uraguchi and Fujiwara 2012). Chronic exposure to cadmium causes an accumulation of cadmium in human kidneys resulting in serious health risks including cancer, osteoporosis, osteomalacia, and cardiovascular disease (Uraguchi and Fujiwara 2012). 90% of cadmium exposure for the non-occupational, non-smoking population is through food (Clemens et al. 2013). The accumulation of cadmium in rice (Oryza sativa) can be attributed to its similarity to other essential metal ions and the use of transporters responsible for their intake (Slamet-Loedin et al. 2015). The ease in which cadmium is taken in by rice, and the large percent of the human population which depends on rice as a staple food, has raised concerns over widespread cadmium exposure (Uraguchi and Fujiwara 2012).

Cadmium, once taken in by rice, is detrimental to its cell health as long as it remains in the cytosol (Mendoza-Cózatl et al. 2011). Toxic trace heavy metals such as Cd have chemical properties similar to micronutrients such as Zn and are generally transported in plants by the same transporters as those micronutrients. There are membrane transporters that are specific for Cd or for Cd-ligand conjugates (Pike et al., 2009). As with micronutrients, Cd content in seeds is affected by processes such as root uptake, xylem translocation, subcellular compartmentalization (e.g. vacuoles), and redistribution via the phloem during seed fill (Figure 1). One of the mechanisms that aid in detoxification is subcellular compartmentalization into the vacuoles. Glutathione (GSH), a tripeptide antioxidant, and phytochelatin (PC), an ogliomer of glutathione (Mendoza-Cózatl et al. 2011) function as chelators which bind to toxic heavy metals (Mendoza-Cózatl et al. 2011). Once bound, these complexes are then transported into the vacuole where they can be safely stored (Mendoza-Cózatl et al. 2011). Due to these vital functions for which GSH and PC are responsible, the study of the relative levels of GSH and PC found in rice may prove beneficial in preventing cadmium accumulation in the rice grain. Heavy metal ATPase3 (HMA3) is a vacuolar membrane transporter that is known to aid in subcellular compartmentalization of Cd in the vacuoles (Figure 2).

The main goal of this study is to study the expression profiling in the different tissues of two different rice lines to monitor expression of a three different genes (PCS1, GSH1, and HMA3) that may influence Cd transport radially across the root, into the vacuoles and/or the loading of Cd into the xylem and phloem.

Materials and Methods

For the purpose of this experiment, two cultivars of rice were chosen from the United States Department of Agriculture Mini-Core collection which exhibited different levels of cadmium accumulations in their grains. Line 310519, from the *indica* subspecies, exhibited higher than average cadmium grain accumulation while line 310428, from the *japonica* subspecies, exhibited lower than average cadmium grain accumulation. Each line was then divided into two groups, half of which were exposed to a 0.5 micro molar cadmium concentration to their roots and the other half served as the control. The rice was grown in hydroponics using a nutrient solution for four weeks. The rice plants were then harvested while still in the vegetative state of development.

Initially, the stems were cut at the base and xylem sap was collected from each of the plants and frozen. This sap will be analyzed for GSH and PC content. Each plant was then separated into leaves, stems, or roots and flash frozen in liquid nitrogen in order to preserve cell conditions at the moment of harvesting. RNA was then extracted from each tissue and a complementary DNA (cDNA) was then constructed through real-time polymerase chain reaction (RT-PCR) using the RNA as a template. This cDNA was then used to conduct a quantitative polymerase chain reaction (qPCR) procedure using custom primers designed to target OsGSHS and OsPCS1 genes, as well as actin, a housekeeping gene. The resulting data would indicate the approximate levels of GSH and PC gene expression at the time of harvest.

Figure 1: The Amount of Cadmium Found in Rice Grains is a Result of Root-to-Shoot Transport Within the Xylem

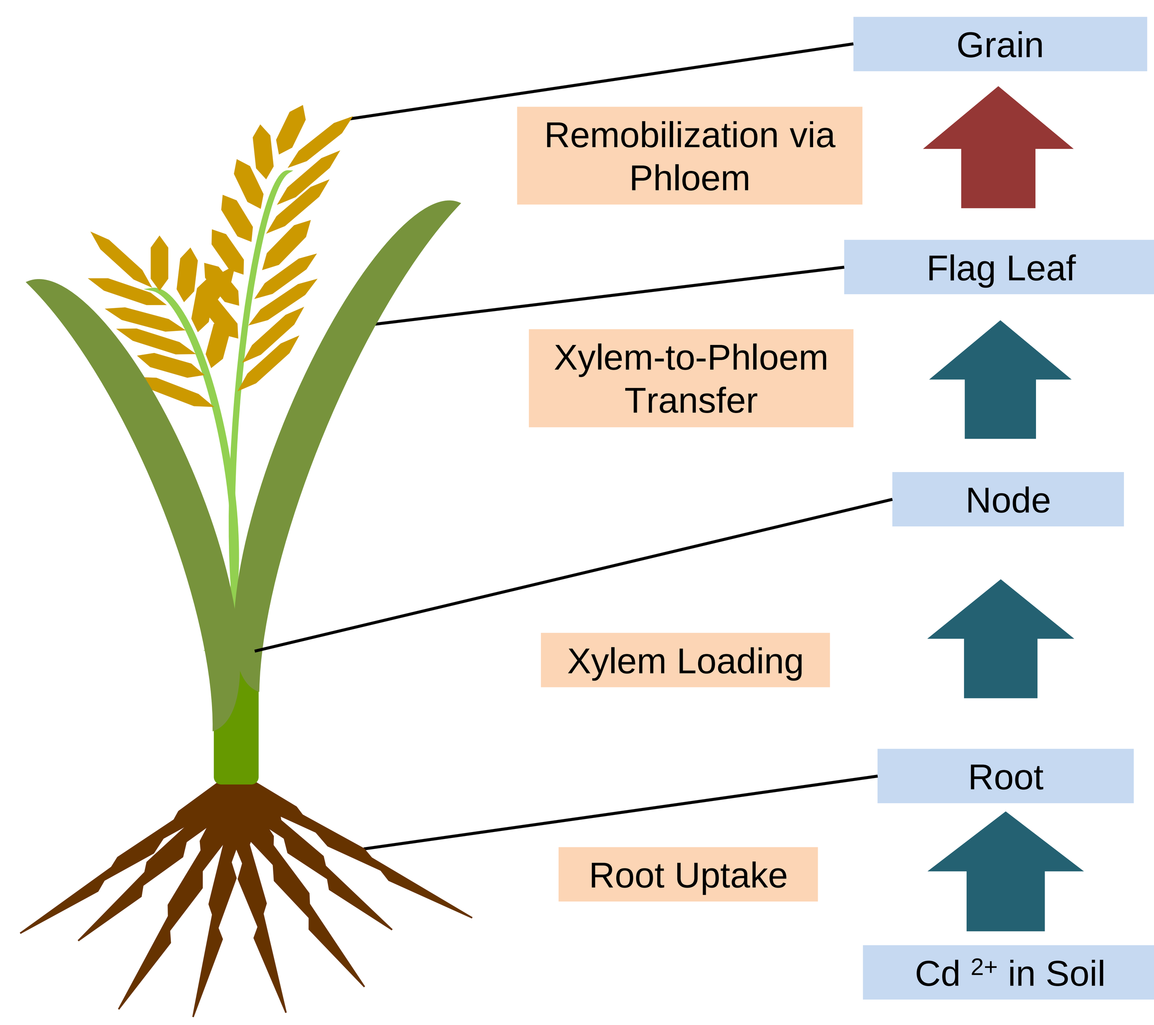
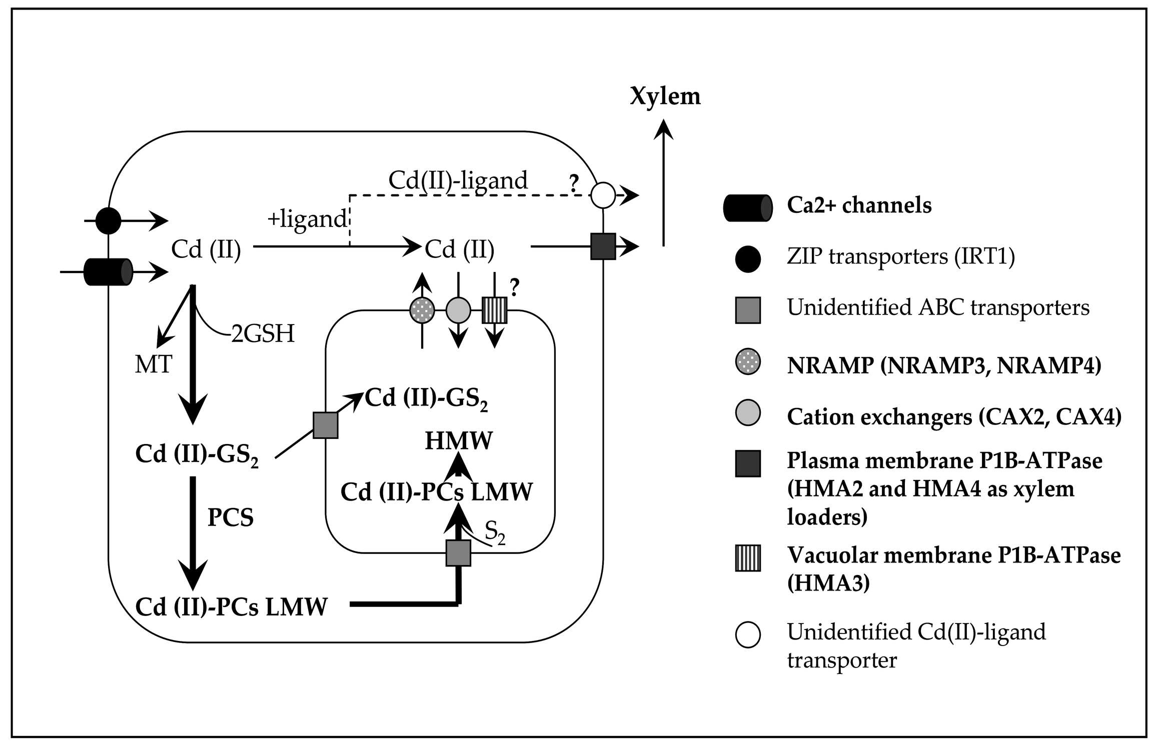


Figure 2: Proposed mechanisms for root intracellular Cd transport (Adapted from Verbruggen et al., 2009).



Results

Figure 3 Relative Chlorophyll Content (RCC) of the rice lines grown in the presence of Cd

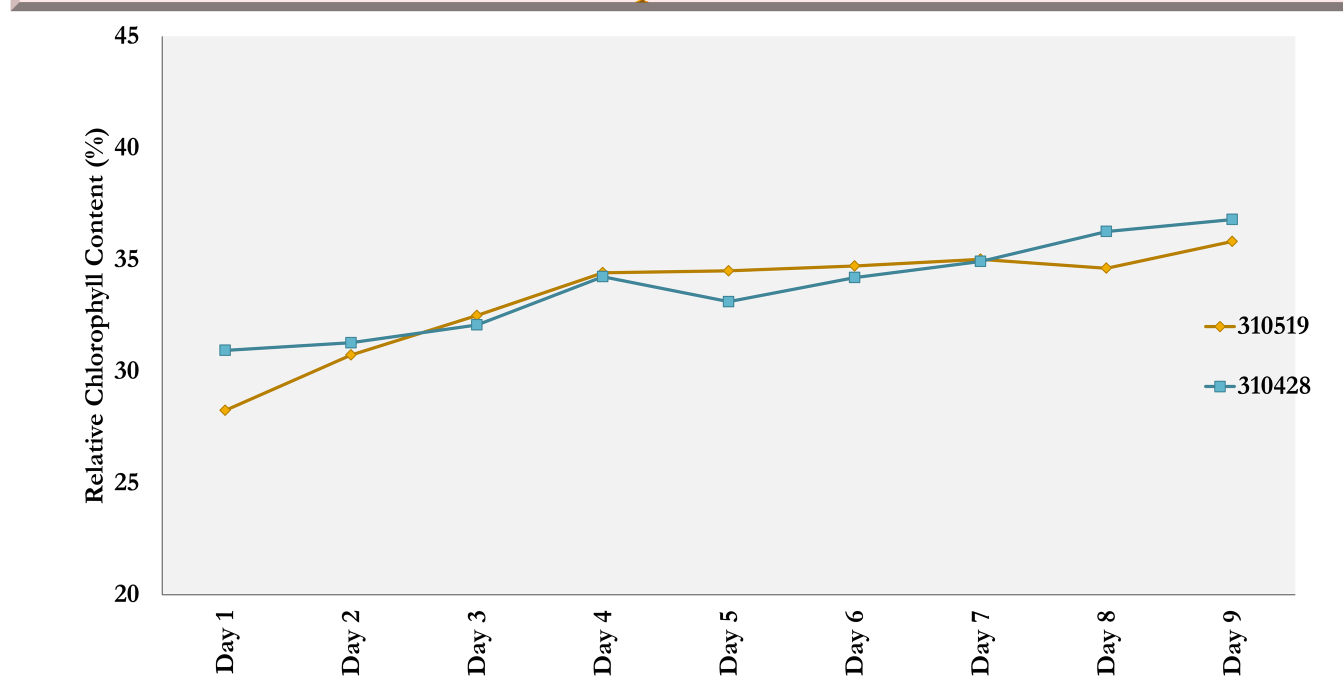
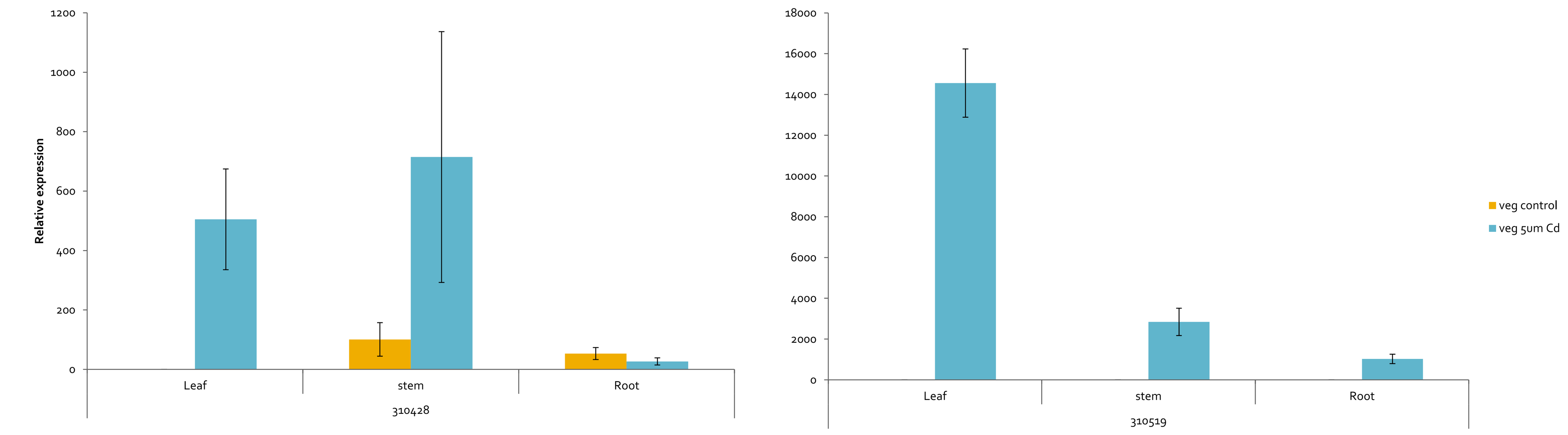
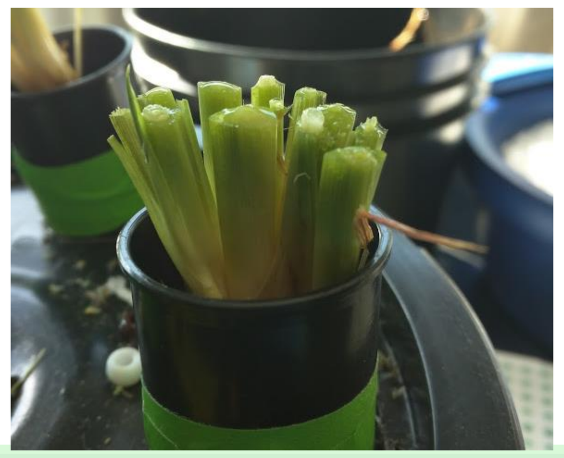


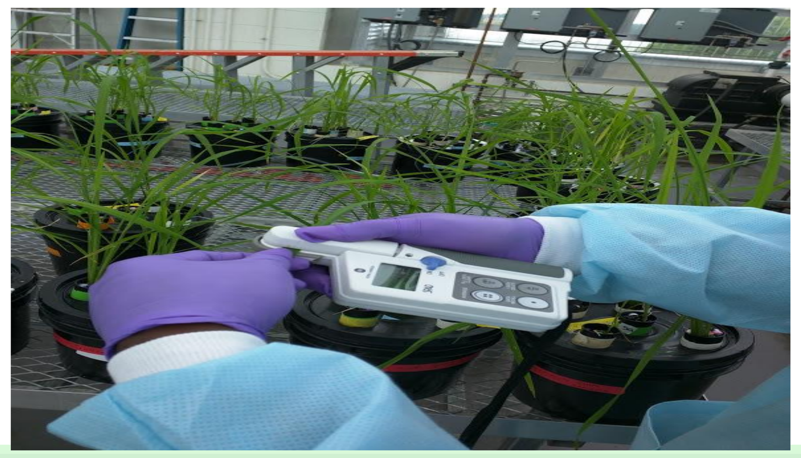
Figure 4 Relative expression of GSH1 in A) 310428 and B) 310519 rice lines during the vegetative stage. Values represent mean ± s.e

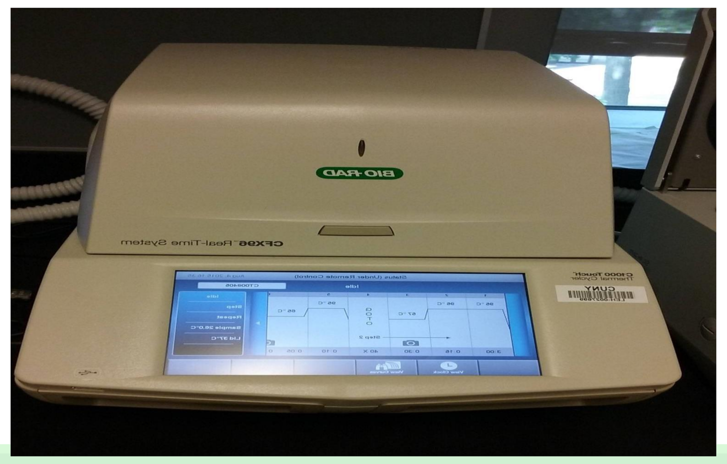


Conclusions

- There was no difference in the RCC between both lines which indicates that cadmium did not have any effect on the physiological well being.
- There was no relative expression of HMA3 in both lines and in both treatments. This indicates that the HMA3 primers might have to be redesigned and relative expression reanalyzed.
- In both lines, GSH expression is higher in the Cd treated plants., Moreover, the expression is higher in the 310519, a high Cd accumulator when compared to 310428, a low Cd accumulator; this indicates that GSH is a possible detoxification mechanism in rice.
- GSH expression is higher in the leaves and stems when compared to the roots which indicates that GSH is produced in all the tissues as a mechanism to chelate Cd.
- PCS expression was not significant in both lines and in both treatments which indicates that the analysis has to be repeated.
- Xylem sap will be analyzed for PC and GSH content.







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