Genes that mediate arsenic and heavy metal detoxification in plants.

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Arsenic is the 20th most abundant element in the earth's crust, and found ubiquitously in nature. Cadmium and arsenic have been reported by the U.S. Environmental Protection Agency to be among the five most toxic substances found at contaminated Superfund sites (Johnson and Derosa, 1995). Although these substances are considered to be among the most toxic substances found at environmentally contaminated sites, little is known about the molecular and genetic mechanisms of plants which survive in habitats containing these metals. Several naturally occurring plants have been identified with increased tolerance to arsenic. Most notable of these plants is the brake fern *Pteris vittata*, which can accumulate over 1% of its dry mass as arsenic (Ma *et al.*, 2001). However, the exact mechanisms utilized by the fern to survive high levels of arsenic are largely unknown.

To gain insight into the mechanisms of arsenic tolerance in plants, we developed a genetic screen to isolate *Arabidopsis thaliana* mutants with altered tolerance to arsenic. We will report on the isolation of a mutant, *ars1*, with increased tolerance to arsenate. *ars1* germinates and develops under conditions that completely inhibit growth of wild type plants and shows a semi-dominant arsenic resistance phenotype. *ars1* accumulates similar levels of arsenic as wild type plants, suggesting that *ars1* plants have an increased ability to detoxify arsenate. However, *ars1* plants produce phytochelatin levels similar to wild type and enhanced resistance is not abolished by the γ-glutamylcysteine synthetase

inhibitor BSO. Furthermore, *ars1* plants do not show resistance to arsenite or other toxic metals such as cadmium and chromium. However, *ars1* plants do have a higher rate of phosphate uptake than wild type plants, and plants grown with an excess of phosphate show increased tolerance to arsenate. Traditional models of arsenate tolerance in plants are based on the suppression of phosphate uptake pathways, and consequently the reduced uptake of arsenate. Our data suggest that arsenate tolerance in *ars1* is due to a new mechanism mediated by increased phosphate uptake in *ars1*. Models discussing how increased phosphate uptake could contribute to arsenate tolerance are discussed.

Work in our laboratory also focuses on examining the roles of phytochelatins, small metal binding peptides produced under exposure to toxic metals. The wheat *TaPCS1* (Phytochelatin Synthase) gene has been shown to mediate heavy metal resistance in *S. cerevisae* (Clemens *et al.*, 1999) and was utilized to explore the possibility of targeting phytochelatins to certain regions of the plant. The goal of these studies is to analyze whether phytochelatin-metal complexes are transported from roots to plant leaves. In the present experiment, the *TaPCS1* gene was targeted to tissues in the Arabidopsis mutant *cad1-3*, which shows no detectable phytochelatin synthesis. Northern and Western analyses confirmed the targeted expression of *TaPCS1* when driven by different promoters. Seedling phenotype analysis showed that both wild type and the transgenic lines analyzed germinated and grew healthy under heavy metal stresses, while *cad1* failed to grow. Data will be presented from Inductively Coupled Plasma (ICP) spectroscopy, HPLC, and Northern and Western analyses towards determining whether phytochelatins move within the plant. The reported results directly address the questions whether the *TaPCS* gene functions in long distance transport and in tissue specific metal homeostasis

when expressed in *Arabidopsis.*

References

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