Final Report for the Project "Preliminary Evaluation of a Genetic Solution to Heavy Metal Risks in Lettuce"

Abstract

Heavy metals are of concern in edible fresh fruits and vegetables. Many of the alluvial soils used for crop production in the Arizona Desert contain low levels of several metals, including lead (Pb) and cadmium (Cd). The principal source of irrigation water for vegetables and fruit crops in the low desert is the Colorado River which also contains detectable levels of these metals. In addition, these metals are commonly found in the phosphate fertilizers widely used in the region. Accumulation of these metal elements into food crops is a health concern as potential carcinogens or causal agents of human organ dysfunction. A previous survey conducted with SCBG funding found 10% of the spinach samples collected exceeded the EU ML of 300 ug/kg fw for Pb and 33% exceeded the EU ML for of 200 ug/kg fw for Cd. Approximately 4% of the leaf lettuce samples also exceeded this ML for Cd. The 95%tile exposure estimates exceed the FDA provisional total tolerable intakes (PTTI) for young children. Overall, the health risks from Pb and Cd from vegetable and fruit crops produced in the low desert are generally low. However, there are a few instances of potential compliance challenges. The objective of this project was to explore a potential genetic solution to the existing and forthcoming heavy metal challenges. This preliminary analysis using the Pavane x Parade recombinant inbred line (RIL) suggest breeding is a likely strategy to reduce heavy metal exposure from leafy vegetables.

Introduction

The production of vegetable and fruit crops in the low desert is over a 2 billion dollar industry. Most of these vegetables are irrigated with Colorado River water which contains low levels of several heavy metals. Consumers of produce are increasingly seeking assurances from industry that their product is safe. Growing trends in monitoring and regulating heavy metals in food are producing similar concerns.

Lead (Pb) has multiple toxic effects on the human body (ASTDR, 1990). Non-carcinogenic effects include decreased intelligence in children (Canfield et al., 2003), increased blood pressure in adults (Schwartz, 1991), kidney impairment, and reproductive effects (Chowdhury et al., 1984). Lead concentrations in the Colorado River water are generally less than 1 μg/L but concentrations in suspended sediments can be as high as40 μg/g (USGS, 2004). Many of the agricultural soils in the region are derived from river sediments deposited before the construction of the network of Dams to manage the river. The WHO has established a Pb RfD of 7 ug/kg body weight-day. The European Union MCL for Pb in leafy vegetables is 300 μg/kg fw (Berg and Licht, 2002). The Center for Disease Control (CDC) has suggested that blood level of10 ug/dL as a level of concern in children. The FDA PTTI are based on estimated projections of pro-longed daily intakes that would raise blood levels in children by 1 ug/dL and dividing by 10 for uncertainties (Carrington and Bolger, 1992).

Cadmium in excessive amounts can cause hypertension, kidney impairment, genetic toxicity, immuntoxicity, neurotoxicity, and carcinogenicity (ATDR, 1997). Cadmium (Cd) is naturally present in many soils and in most phosphate fertilizers (Mortvedt et al., 1981). Food is the major source of Cd exposure to humans (Gunderson, 1995; Pennington et al., 1986). The World Health Organization (WHO) has established a provisional daily intake of cadmium at 1 μg/kg body weight (Walker and Herman, 2000). Based on consumption estimates and cumulative exposure projections, the EU has recommended maximum levels (MLs) for various food commodities. For example, the ML for fruits, rooting vegetables, wheat, and leafy vegetables are 50, 100, 200, and 200 µg/kg fw Cd, respectively (Berg and Licht, 2002). The levels of Cd in Colorado River water are generally less than 1 µg/L. However, we have found levels of Cd in phosphate fertilizers used in the low desert as high as 150 mg/kg. Work in the California central coast has shown the potential for Zn fertilization to reduce Cd uptake by crops. The Durum wheat industry in Arizona has also eliminated Cd compliance issues by breeding wheat cultivars with low Cd transfer to the grain. Other researchers have suggested breeding as a strategy to reduce heavy metal exposure in food crops (Grant et al., 2008; Clemens et al., 2012), including leafy vegetables (Wang et al., 2007).

In a previous survey funded by the Arizona specialty crop block grant program we found accumulations of heavy metals were generally higher in edible leafy vegetables, such as lettuce and spinach, compared to fruiting crops such as citrus, tomato, and dates. However, with the exception of spinach and leaf lettuce, most products sampled were generally below EU MLs for Pb and Cd. The DEEM (Dietary Exposure Evaluation Model) model was used to derive an estimate of the 2-day average intakes of these metals in food and water. Drinking Colorado River water was found not to be a significant relative source for Pb and Cd . The cumulative exposures from all fruits and vegetables to Pb and Cd, and As, were always less than 5, and 10%, their respective RfDs. The 95%tile exposure estimates, but not the mean exposure estimates for lead, do exceed the FDA PTTI for young children.

The FDA is currently considering the regulation of heavy metals in fresh produce. Because levels of heavy metals in our soils and irrigation water may present potential compliance issues, work is needed to develop management strategies aimed to reduce metal exposure in produce. Recently, the SCBG program approved a project to evaluate soil testing as a tool to manage heavy metal exposure through Arizona vegetables. The objectives of the project proposed here is to evaluate a potential genetic solution to the heavy metal issue in lettuce.

Materials and Methods

Field studies were conducted using a RILs we have identified (Pavane x Parade). This recombinant inbred line population of lettuce has genetic linkage maps suitable for QTL (quantitative trait loci) analysis. The field site was mapped as a Casa Grande Loam (Fine-loamy, mixed, superactive, hyperthermic Typic Natrargids, reclaimed) located at the Maricopa Agricultural Center. Tissue samples were collected from all lines, ground, digested, and analyzed for Cd, and Pb using inductively plasma mass spectroscopy. These data were then be subjected to QTL analysis by the USDA-ARS Laboratory in Salinas California to identify genes or combinations of genes that reduce heavy metal accumulation.

Results

The variation in Cd and Pb concentrations across lines for the RIL utilized are shown in Figures 1 and 2. The results of QTL analysis show a strong QTL for Pb tissue concentration (Table 1). Although there were no significant QTL for Cd concentration at P=0.05 there was a suggestive QTL at P=0.1 (Lander and Kruglyak, 1995). Since the calculation of the corrected point (nominal) p-value or LOD score threshold involves considering the number of chromosomes, genome size (in cM), phenotype fluctuation and experimental design, we could not apply the methods used by Lander and Kruglyak to our population and cadmium data. We used the guidelines they suggest for genome-wide significance levels (Significant QTL α= 0.05; Suggestive QTL α =1) and applied a Bonferroni correction of the number of markers. We compared the threshold level (0.05) of the Bonferroni correction (3.691) to the more commonly used 1000 permutation test (3.46), which is based on the specific data set we are looking at. Thus, for Cd concentration two suggestive QTLs arise using this threshold (Table 2 and Figure 3). Whether this suggestive QTLs have true biological importance need to be tested by extending the study with additional experiments either use this RIL or a large one.

Overall, these data provide evidence that plant breeding may be a viable strategy to reduce heavy metal exposure from leafy vegetables.

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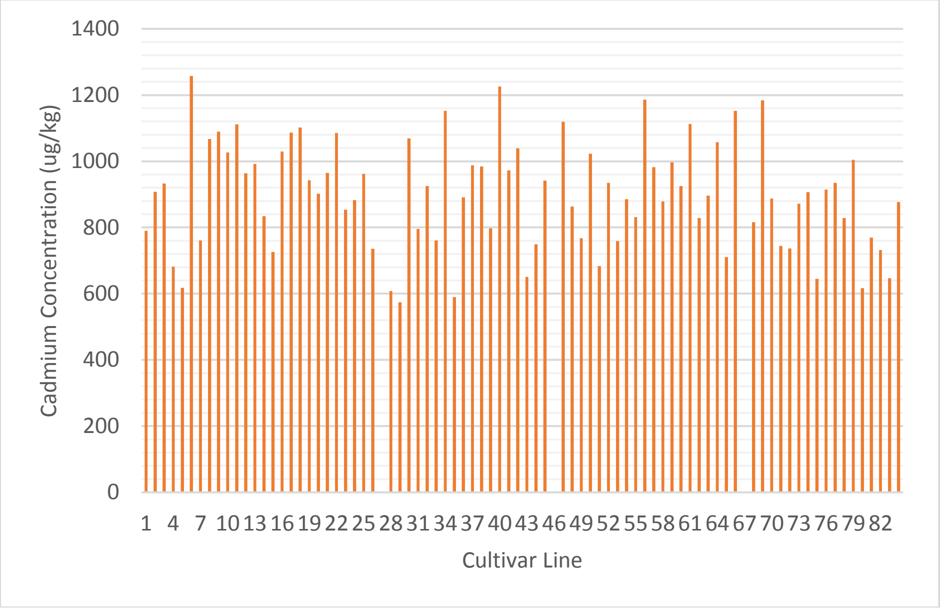


Figure 1. Variation in cadmium (Cd) concentration in edible lettuce leaves across recombinant inbred lines.

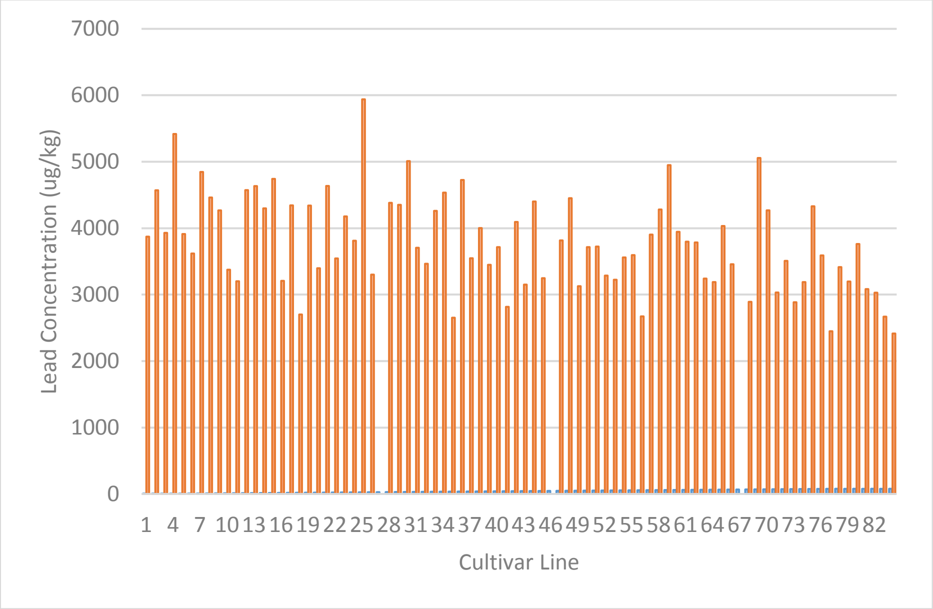


Figure 2. Variation in lead (Pb) concentration in edible lettuce leaves across recombinant inbred lines.

Table 1. Significant QTL for Pb concentration in lettuce tissue.

Experiment	Trait	LG	position	A	(p-value)	R2 (h^2)	SMR(LOD)	CIM(LOD)
HM	Pb ug/kg	4a	0	273.1408	0.000108	0.179	3.25	3.25

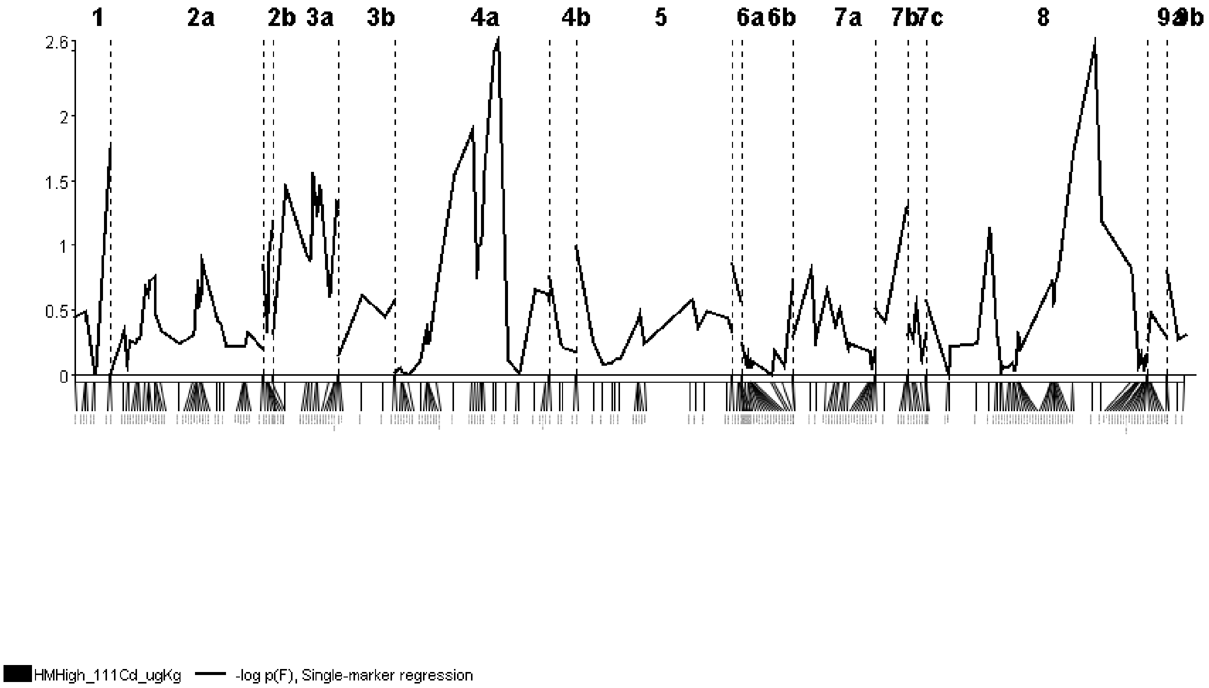
Table 2 and Figure 3.

Comparisons of corrected thresholds for a single test (single marker) for Cd concentration by different methods

		Significant QTL	Suggestive QTL
Genome wide		α= 0.05	α= 1
Bonferroni correction for number of markers (number of comparisons)	-log(p)	3.691	2.39
Bonferroni correction for cM genome size	-log(p)	4.275	2.9
Range suggested in paper (mouse genome)	-log(p)	4-5	3-4
	LOD	3.3-4.33	1.9-2.8
By 1000 permutations (qgene) on Cd(ug/Kg) data	-log(p)	3.46	NA
	LOD	2.726	NA

For the Cd (ug/Kg) data two suggestive QTLs arise using this threshold.

Linkage group	position	-log p(F)	LOD
4a	73.5	2.57	2.035
8	120.9	2.537	1.997



X-axis: markers along the genome, linkage group names noted on top of the graph. Perpendicular

dashed lines separate linkage groups.
Y-axis: -log p(F) for Cd (ug/Kg)