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Mutant Mapping of Arabidopsis Lines Displaying Enhanced Responses to Cadmium, and Monitoring Heavy Metals and Metalloids in Crops Produced from Campus Community Gardens

A Thesis submitted in partial satisfaction of the requirements

for the degree Master of Science

in

Biology

by

Alexander Scavo

Committee in charge:

Professor Julian I. Schroeder, Chair
Professor Steven Briggs
Professor Eric Schmelz

2019

The Thesis of Alexander Scavo is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

2019

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ACKNOWLEDGEMENTS

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Special thanks to Qi Yu for his continuous support and friendship, and to Yasman and Maggie who will be pushing this project to new heights as I finish my part in this story of heavy metal research.

ABSTRACT OF THE THESIS

Mutant Mapping of Arabidopsis Lines Displaying Enhanced Responses to Cadmium, and Monitoring Heavy Metals and Metalloids in Crops produced from Campus Community Gardens

by

Alexander Scavo

Master of Science in Biology

University of California San Diego, 2019

Professor Julian Schroeder, Chair

Heavy metals and metalloids are a prominent threat to human health worldwide. Unfortunately, plants expose people to heavy metals and metalloids through diet. To develop plants capable of protecting people from heavy metal and metalloid exposure, the molecular systems of plants in their response to heavy metals and metalloids must be elucidated. In the first chapter of this study, a luciferase reporter construct containing a promotor region for the *SULTR1;2* gene was inserted into *Arabidopsis thaliana* lines. These lines were then mutated with Ethane methosulfornate (EMS), and a forward genetic screen was conducted that searched for

shifts in luciferase luminescence in response to cadmium. In these screens, 3 types of classes of shifts in luminescence were procured from the mutant lines, and were classified as having a constitutive (*crc1*), super (*src1*), or non-response (*nrc1, 2*) to cadmium. The *nrc1, 2* mutants have since been characterized. Using bulk-segregation analysis, mutated genomic regions that separated with the *src1* and *crc1* luciferase phenotypes were determined. In addition, the *crc1* mutants expressed a root-growth phenotype in sulfur- free media supplemented with cadmium and selenium. *Src1* mutants showed a greater *SULTR 1;2* expression in response to cadmium. T-DNA knockout lines for all candidate genes were ordered, genotyped, and propagated, and were used to determine potential causative genes for the *src1* and *crc1* phenotypes. In the second chapter, inductively-coupled electron optical electron spectroscopy was conducted to determine the heavy metal and arsenic concentrations of edible crop tissues grown in Campus Community Gardens around UCSD.

I.

Mutant Mapping of Arabidopsis Lines Displaying Enhanced Responses to Cadmium

1.1: Abstract

Understanding the molecular mechanisms used by plants to both transport and sequester heavy metals from the environment can have promising applications in agriculture, ecology, and human health. For important crop varieties, research is aimed at preventing heavy metals and metalloids from entering edible tissues. For plants engineered to remediate contaminated soils, research is conducted to develop plants able to absorb larger amounts of heavy metals and metalloids from the environment.

In this chapter, we attempt to achieve a new understanding of the molecular interactions between plants and the toxic heavy metal cadmium. To accomplish this, a forward genetic screen was conducted by transforming *Arabidopsis thaliana* with a luciferase reporter construct, and then mutagenizing these lines with EMS. These mutants were then screened for shifts in their response to cadmium in the M3 generation. Three classes of shifts in cadmium response were obtained, and were classified as having a super response to cadmium (*src1*), constitutive response to cadmium (*crc1*), and a non-response to cadmium (*nrc1*). Prior to this study, the causative mutations for the non-response to cadmium were characterized. Candidate regions containing the mutations responsible for the *crc1* and *src1* phenotypes were determined by using bulk-segregation genome resequencing analysis. T-DNA lines were ordered, propagated, and subsequently genotyped for all candidate genes. Root-growth assays, luciferase imaging, and RT-PCR were conducted in order to determine potentially causative genes for both the *crc1* and *src1* phenotypes.

1.2: Introduction

Human exposure to heavy metals and metalloids reached its highest extent after the world had emerged from the industrial age, and is an occurrence that is truly as old as civilization itself (Industrial age anthropogenic inputs of heavy metals into the pedosphere). Today, heavy metals and metalloids continue to exist in the environment worldwide due to a plethora of anthropogenic means, especially those pertaining to mining, agriculture, energy storage and production, irrigation, and biological waste management. Interestingly, heavy metals and metalloids are also a natural component of earth's crust, and eventually migrate to mantel soil layers (Alloway, 2013)

Currently, the Agency for Toxic Substances and Disease Registry (ATSDR)- a contingent of the Center for Disease Control and prevention (CDC)- has developed a "Substance Priority List" which rates 275 toxic compounds based on three criteria: 1) How frequently the compound is found at priority sites, 2) How toxic the compound is, and 3) The potential for this compound to come into contact with large amounts of people. On this list, heavy metals dominate the top ten compounds, of which cadmium is included (CERCLA 2015).

Cadmium has been shown to be a major component in the onset of end-stage renal disease, where the kidneys of the affected had been reduced to about 10% functionality compared to kidneys devoid of chronic cadmium toxicity (Tellez-Plaza et al., 2013). Furthermore, elevated urine cadmium levels- a common method of determining cadmium accumulation in humans- has been shown to be implicated in major coronary heart disease, stroke, and heart failure (Tellez-Plaza et al., 2013; Ujueta et al., 2018). Amongst patients with

Alzheimer's disease, those with elevated blood cadmium levels greater than 0.6ug/L where shown to developed almost four-fold higher mortality rates when compared to a control populace with blood cadmium concentrations lower than 0.3ug/L (Min & Min, 2016). Indeed, emerging studies are for the first time, directly linking cadmium to a series of neurological disorders, including neuronal cell death, and the accumulation of Tau and Amyloid plaques in the brain. (Chen, Liu, & Huang, 2008; López, Figueroa, Oset-Gasque, & González, 2003). From 1910-1960, drainage from a zinc mine in the Jinzu River basin in Toyama, Japan, caused extensive cadmium contamination of soil and local groundwater resources. This resulted in the first-ever diagnosis of the *Itai-Itai* disease: a form of renal tubular osteopmalacia, resulting in massive kidney damage, and agonizing bone malformations. These diseases have since been directly related to cadmium contamination, with the severity correlating to the level of cadmium present in the environment (Aoshima, 2012).

While cadmium exposure can occur from drinking water and occupation, most of it arises through the consumption of plants grown in contaminated soils. However, there are many factors that affect plant cadmium concentrations, such as the species of plant, salinity of the soil, and surrounding environmental pH. Currently, cadmium is known to be most absorbed by cereals, cacao, and leafy vegetables (Joint, F. A. O., World Health Organization, 2006). There are currently many strategies developed aimed at reducing cadmium uptake by plants, and often revolve around adding expensive (organic and nonorganic) soil amendments to crops, breeding cultivars that are naturally devoid of cadmium uptake from the soil, and water management and irrigation practices (Ali et al., 2017).

Much research has been conducted on elucidating the molecular mechanisms by which plant root systems take up heavy metals, especially cadmium and arsenic (Clemens & Ma, 2016).

In the root cells of plants, several metal transporters exist with the function of transporting exogenous metal elements into the cell. However, many of these transports, including the Iron Regulated Transporter (IRT), have an affinity to bind and transport many different metal elements, including cadmium (Korshunova, 1999; Thomine, Wang, Ward, & Crawford, Nigel M. and Schroeder, 2000). In addition, several ATPases and Cation Diffusor Facilitator (CDF) proteins have been found to sequester and/or transport cadmium throughout several intracellular compartments in plant cells and are thought to be involved in heavy metal homeostasis (Emerging mechanisms for heavy metal transport in plants). Of the ATPases, HMA type P have been heavily implicated in root-to-shoot cadmium transportation. Indeed, mutant *Arabidopsis Thaliana* knockout HMA2 and HMA4 lines crippled cadmium root-to-shoot translocation from 60% to 2% of total cadmium absorbed (Hanikenne et al., 2008; Wong & Cobbett, 2009).

While transport proteins are essential to maintain plant heavy metal homeostasis, phytochelatin proteins, synthesized by phytochelatin synthases, are crucial for plant heavy metal resistance, and can sequester cadmium from the cell cytosol into the vacuoles of plants (Cobbett, 2002). Additionally, phytochelatins have been implicated in long-distance transportation of cadmium, and have drastic affects on cadmium mobility via xylem tissues (Gong, Lee, & Schroeder, 2003). Phytochelatins are composed of glutathione precursor molecules, in which they themselves contain several cysteine rich sulfur groups, and are heavily reliant on the plant's ability to obtain inorganic sulfur (Grill, Loffler, & Zenk, 1989). Currently, the sulfur transporter SULTR 1;2 has been characterized as being an essential component in the root- uptake and mediation of inorganic sulfur from the environment (Nakako Shibagaki, Alan Rose, 2002).

Previously in the Schroeder Laboratory, a luciferase reporter gene was constructed by fusing the promoter region of the *SULTR1;2* gene to the firefly luciferase gene (Jobe et. al.,

2012). This *pSULTR1; 2::LUC* reporter gene was then cloned into *Arabidopsis thaliana,* which were then subsequently mutated with ethyl methane sulfonate (EMS). A forward genetic screen was then conducted to analyze shifts in luciferase luminescence amongst these mutated lines in response to cadmium. Three shifts in luminescence emerged amongst the mutant population, in which were classified as having a super-response to cadmium (*src1*), constitutive response to cadmium (*crc1*), and non-response to cadmium (*nrc1-2*) (Jobe et al., 2012). In the present research for the *src1* and *crc1* mutants a genomic, sequence-based bulk segregation analysis was conducted, and connected the shifts in luminescence (and thus response to cadmium) to a region spanning 15 genes for the *crc1* mutants, and 14 for the *src1* mutants. For the candidate mutations thought responsible for these shifts in the cadmium response, T-DNA knockout lines were ordered, genotyped, and propagated. Interestingly, the *crc1* mutagenized line also displayed a root growth phenotype in -S +Cd +Se media (Cooper, 2018). The corresponding *crc1* linked T-DNA knockout lines were grown in the same media and measured to determine whether comparable root length between T-DNA seedlings and the *crc1* mutant lines could be identified. This was done to select potential causative genes from the candidate region. Unlike the *crc1* mutants, the *src1* lines did not show any root growth phenotype. Instead, reverse -transcription coupled to polymerase chain reaction (RT-PCR) was pursued in order to visualize the relative expression of the *SULTR1;2* gene between the *src1* mutants and the reporter- gene control, in the presence or absence of cadmium. RT-PCR was then conducted on individual T-DNA knockout lines under the same conditions, to determine if gene expression of the candidate genes matched that of the *src1* mutant phenotype.

1.3. Materials and Methods

Plant material and prospective growth conditions

The T-DNA insertion lines were obtained from the Arabidopsis Biological Resource Center (ABRC) at Ohio State University, and were subsequently genotyped to ensure homozygous knockouts were obtained. Seeds were sterilized with 33% bleach and 0.05% Tween-20, and were then vernalized without light at 4˚C at for 72 hours. After being plated in media, seeds were grown in a growth chamber with 12-h light/12-h dark conditions at 21˚C.

Reverse Transciption- Polymerase Chain Reaction (RT-PCR)

For all RT-PCR experiments, seedlings were grown on ½ Murashige and Skoog (MS) medium for 14 days in a growth chamber (conditions listed above). Seedlings were then transferred to either fresh ½ MS control media, or ½ MS media that contained 100 um cadmium chloride, for 6 hours. All MS media contained 1mM MES, an ajustment of pH to 5.6 with KOH, and an agar concentration of 1.5%. 100mg of seedling tissue was collected for each sample, frozen in liquid nitrogen, and ground with 2.3mm steel beads. Seedlings were then processed for RNA extraction using a Sigma-Aldrich Spectrum Total Plant RNA Kit after being ground in a Retsch MM 400 Mixer Mill for 2 minutes at 27 revolutions per second. Subsequently, 3 ug of extracted RNA was purified using a TURBO DNA-free kit from Life Technologies, after total RNA concentration was measured with a Thermo Scientific NanoDrop 1000 spectrophotometer. After unwanted DNA had been remove and DNase deactivated, cDNA synthesis was performed on all samples using the Quantabio qScript cDNA SuperMix kit. 17 cycles were performed for the PCR.

Root Growth Assays

All sulfur-free media implemented was formulated with the following:, 1 mM KH_2PO_4, 0.25 mM $Ca(NO_3)_2$, 30 μM H_3BO_3,10 μM $MnCl_2$, 0.1 μM $(NH_4)_6Mo7O_4$, 1 μM $CuCl_2$,1 μM $ZnCl_2$, , 50 μM KCl,1 mM $MgCl_2$, 100 μM NaFeEDTA, 0.05 mM KNO_3, and 0.5 mM $MgSO_4$. The pH of all sulfur-free media was adjusted to a range of 5.6-5.7, and was done using KOH. Agar concentration was 1.5%. Seedlings were first grown in sulfur-free media for 5-7 days, and were subsequently moved to control sulfur-free media, or treatment sulfur-free media containing 75uM Cadmium, and 1.5uM Selenium, for another 5-7 days. Roots were measured with Image J.

Luciferase Assays

For luciferase imaging, seedlings were grown on ¼ MS media containing 1% sucrose, for 5 days. Seedlings of similar root length were then transferred ¼ MS plates containing 1% sucrose atop 100uM nylon mesh and were allowed to acclimate for a day in a growth chamber with conditions of 12-h light/12-h dark at 21˚C. Next, the mesh and seedlings were transferred to ¼ MS plates with 1% sucrose that had 100uL of 100uM luciferin applied across the surface, and remained so overnight in the growth chamber. Next, the seedlings- atop the nylon mesh- were moved to either control ¼ MS 1% sucrose, or ¼ MS 1% sucrose treatment plates containing 100uM cadmium. Both control and treatment plates, prior to seedling addition, were supplemented with 100uL of 100mM luciferin atop the plates. After this, all plates were incubated for 4-6 hours. Once all plates contained luciferin, regardless if seedlings were added or not, they were incubated in the dark, which was done by wrapping plates in alumium foil. After 4-6 hours, the seedlings were analyzied with a 2 minute exposure rate, and illuminescence was quantified, with a BERTHOLD Night OWL LB981 imaging system.

1.4: Results

Super Response to Cadmium (src1)

As the name implies, the super resonse to cadmium (*src1*) muntants have an increased response to cadmium, relative to the columbia wildtype control that contains the inserted luciferase reporter construct (Figure 1.2). Previously, Andrew Cooper conducted a bulk segragation analysis on the *src1* mutants, and was able to identify a region of 14 genes that consistantly segragated with the increased illuminescence phenotype of the *src1* mutants in luciferase assays (Figure 1.1 and Table 1.1). Since no other phenotype has yet been discovered for the *src1* mutants, reverse transcription coupled to a polymerase chain reaction (RT-PCR) was conducted on the *src1* mutants, as well as the reporter control, to visualize differential *SULTR1;2* expression in the presence or absence of 100uM cadmium. For the *src1* mutants, exposure to cadmium elicited a greater *SULTR1;2* gene response relative to the reporter control (Figure 1.3A). In addition, RT-PCR was conducted on T-DNA lines in order to identify potential candidate genes responsible for the enhanced *SULTR1;2* expression in the *src1* mutants (Figure 1.3B, Figure 1.4). Regarding the RT-PCR for the T-DNA lines, line 4 corresponds to an insert mutation in At4g16267 , 5 corresponds to another T-DNA insert in a different location in At4g16267, and 10 corresponds to corresponds to an insert in At4g15230 (Figure 1.3B). All three of these T-DNA knockout lines showed enhanced *SULTR1;2* expression.

Constitutive Response to Cadmium (crc1)

Like the *src1* mutants, Andrew Cooper conducted a bulk segregation analysis and determined a region on 14 genes to be associated with the *crc1* luciferase phenotype (Figure 1.5, Table 1.2). Since *crc1* mutants displayed a long-root phenotype in the presence of sulfur-free

media that had been supplemented with 75uM Cadmium and 1uM Selenium (Figure 1.6), T-DNA lines were tested to see if there exited a knockout that could recreate this phenotype. To test this, T-DNA lines were ordered by Andrew Cooper, and were bulked and subsequently genotyped to ensure homozygous knockouts by myself (Table 1.2, Figure 1.6). For the *crc1* mutants, genes At4g10930 (T-DNA lines 067394, and 120184) and At4g13575 (T-DNA lines 117073 and 117071) had root-growth phenotypes that mirrored that of the *crc1* mutants (Figure 1.6).

Upon obtaining the *crc1* mutant knockout lines, seed quality was not optimal to continue experiments (figure 1.7A). To overcome this, seeds that were of better quality were sequestered from the pool of *crc1* mutants, and were subsequently tested to ensure that the *crc1* phenotype could still be obtainable (figure 1.7B). In addition, luciferase imaging was conducted to ensure that the report gene construct was still in these mutants (Figure 1.8). Thus, selected *crc1* plants were able to show the root growth phenotype in the presence of 75 uM cadmium and 1uM selenium, and displayed the constitutive illuminance phenotype in luciferase assays. All primers used for genotyping and RT-PCR are shown in table 1.3.

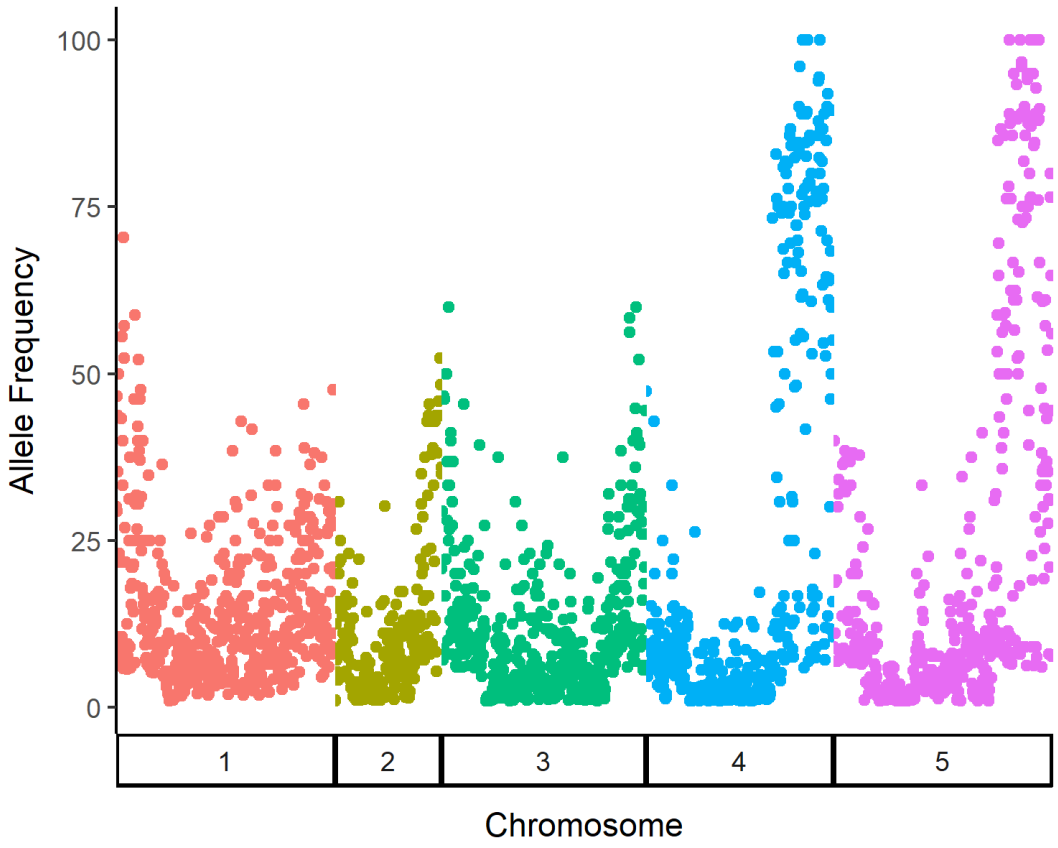

Figure 1.1: Bulk segragation analysis revealing allele frequency associated with the *src1* constituitive luminesence phenotype, done by crossing *src1* mutant populations with bur-0 wildtype, revealing a cadidate region at the end of chromosome 4 in the F2 population. This analysis was performed by Andrew Cooper.

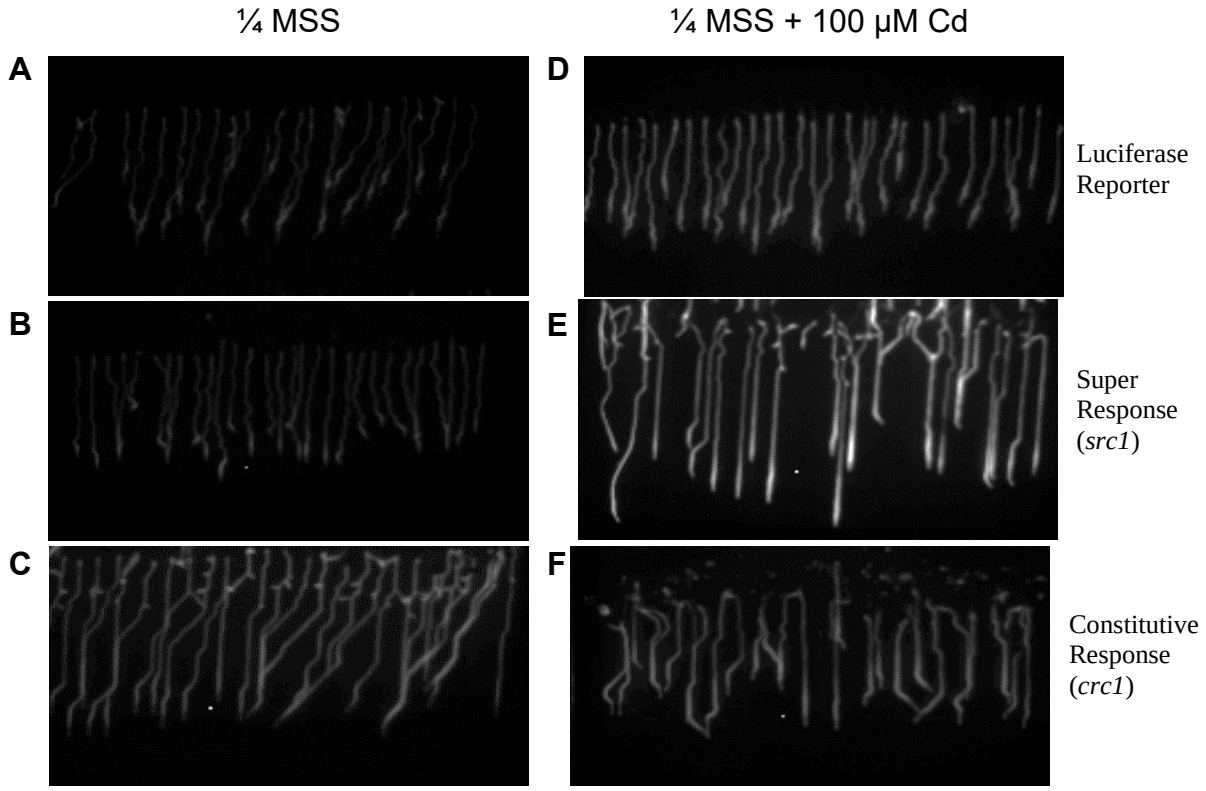

Figure 1.2: Luciferase phenotype of the *src1* and *crc1* mutants, performed by Andrew Cooper. Panels **A)**, **B)**, and **C)** show the reporter line, src1 mutant, and crc1 mutant response to a cadmium free environment. Panels **D)**, **E)**, and **F)** show the same lines but in response to ¼ MSS containing cadmium.

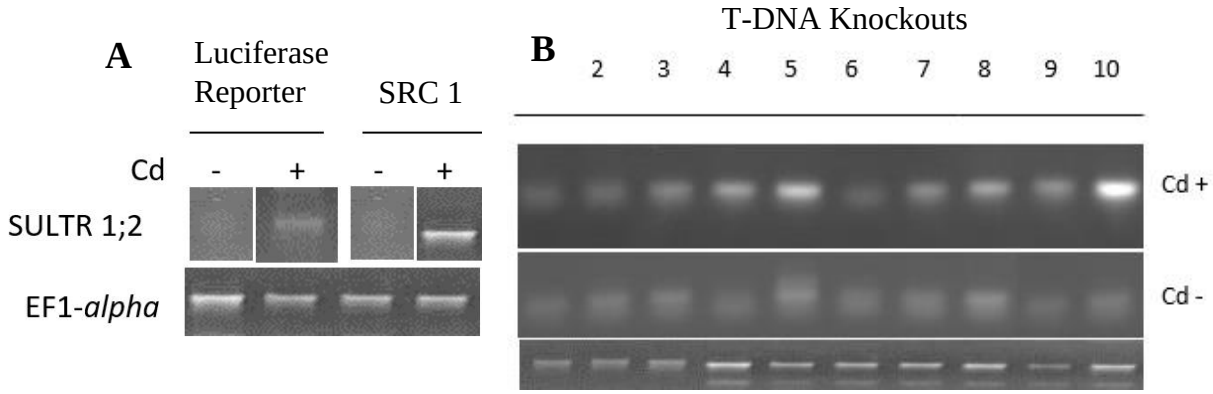

Figure 1.3: RT-PCR of the *src1* mutants, Luciferase reporter control, and T-DNA knockout lines. Elongation factor 1 alpha was used as a loading control. **A)** RT-PCR of the luciferase reporter control and *src1* in the presence and absence of cadmium. **B)** RT-PCR of T-DNA knockout lines in the presence and absence of cadmium. Before RNA extraction, seedlings were either moved to fresh control ½ MS plates, or ½ MS plates containing 100uM of $CdCl_2$, for 6 hours.

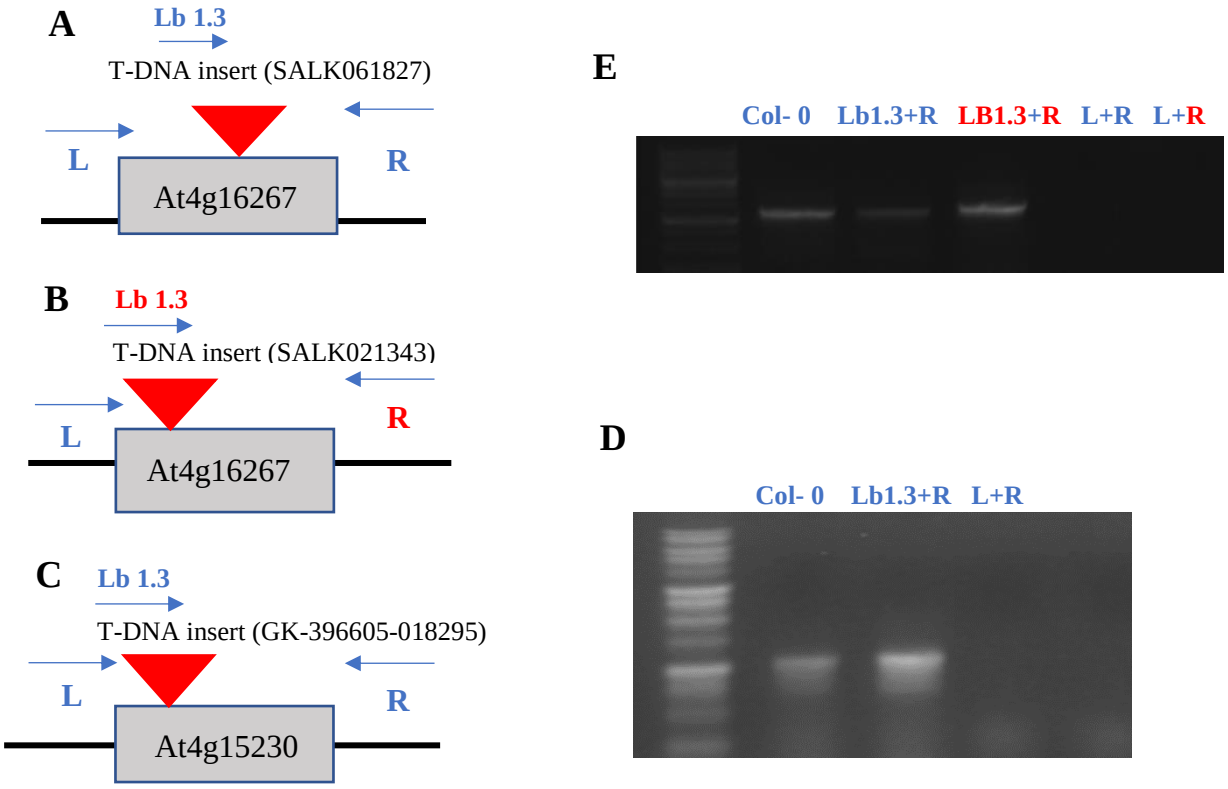

Figure 1.4: At4g16267 has two T-DNA inserts and At4g15230 has one. **A)** and **B)**: schematic representations of T-DNA inserts for At4g16267. **C)** schematic representation for a T-DNA insert in At4g15230. **E)** Genotyping for the At4g16267 inserts. **D)** Genotyping for the At4g15230 insert.

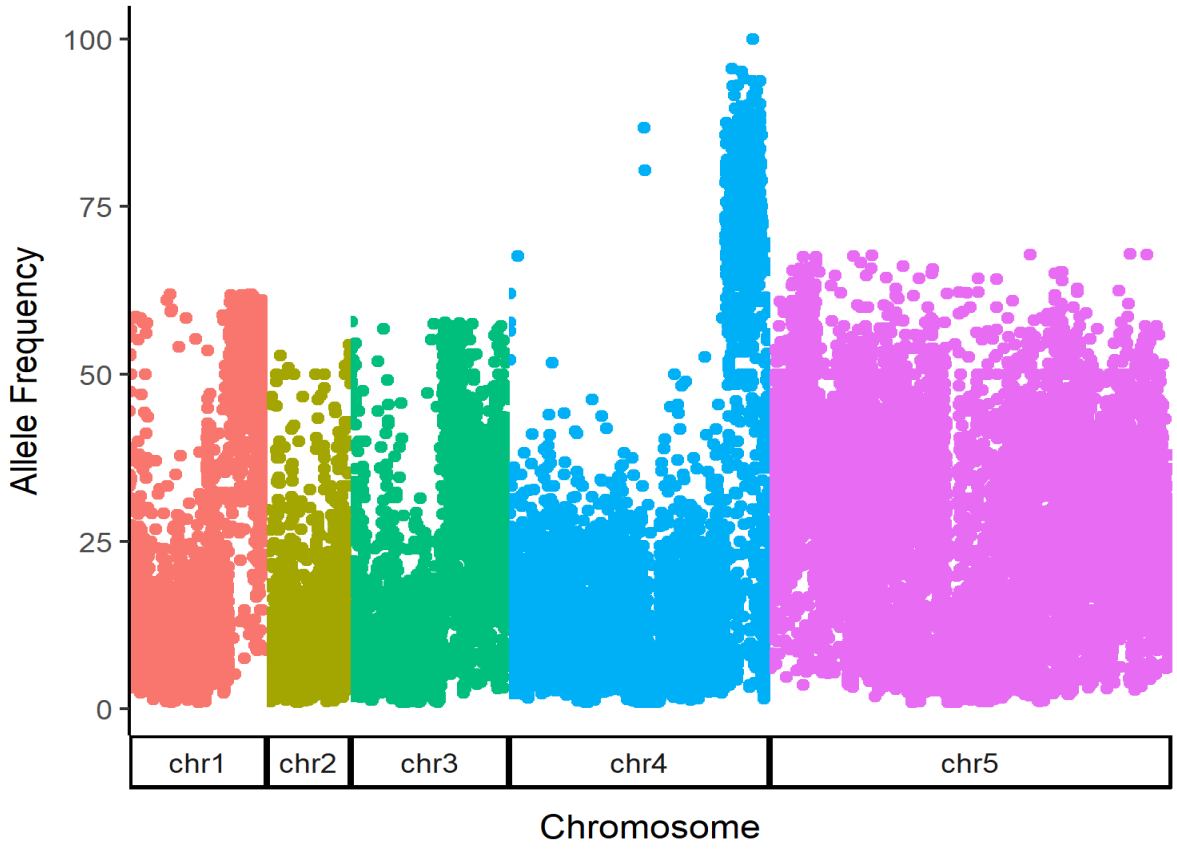

Figure 1.5: Bulk segragation analysis revealing allele frequency associated with the *crc1* constituitive luminesence phenotype. *Crc1* mutant populations were crossed with the luciferase reporter line, revealing a cadidate region at the end of chromosome 4 in the F4 population. This analysis was performed by Andrew Cooper.

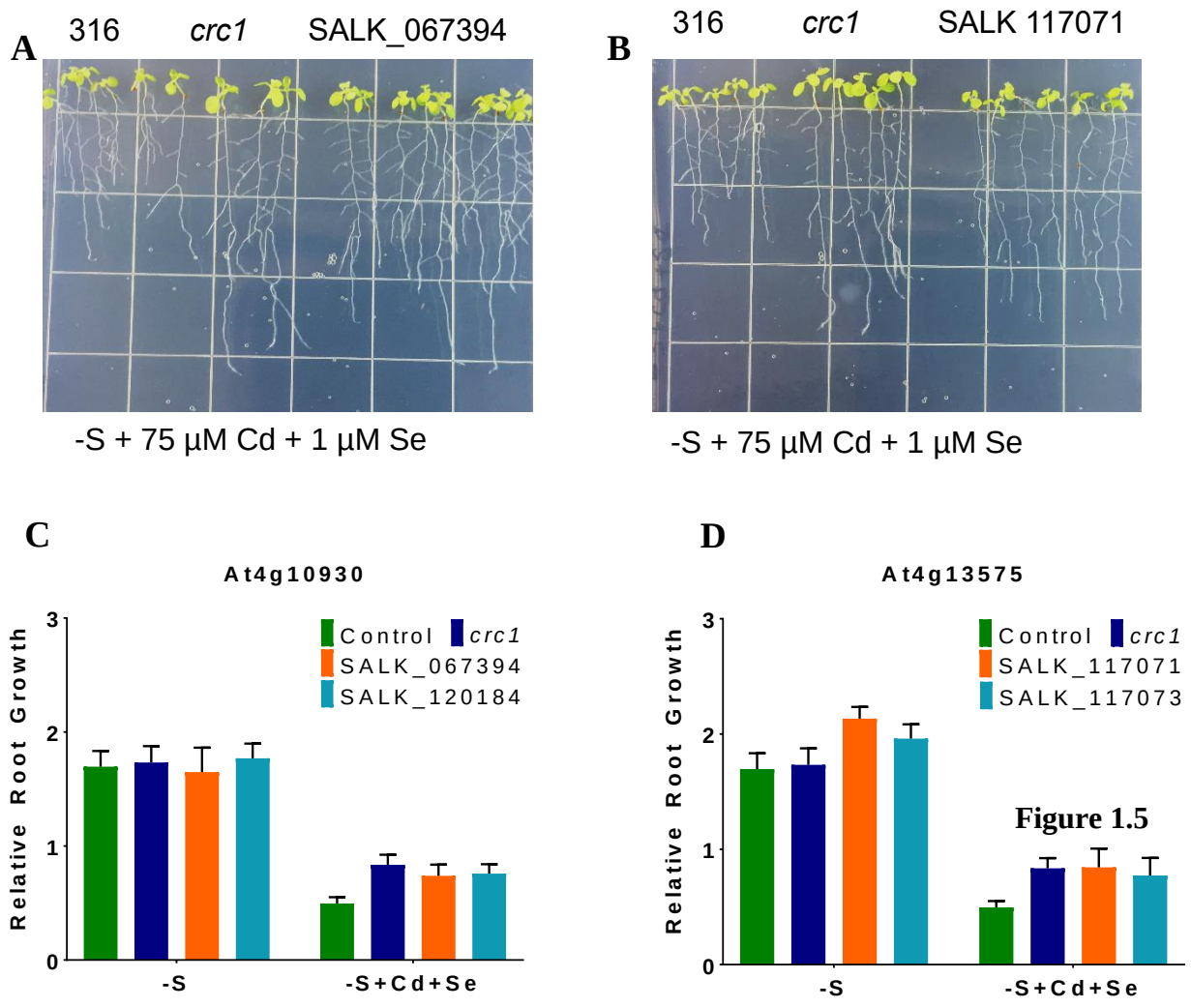

Figure 1.6: *crc1* root-growth phenotype mirrors that of At4g10930 and At4g13575, performed by Andrew Cooper. **A),** and **B)** *crc1* shows longer roots than that of the luciferase reporter control in -S + Cd + Se media. At4g10930 and At4g13575 also display this phenotype. Seedlings were allowed to grow in growth chamber conditions for 7 days in -S media before being plated on fresh -S control plates or -S + Cd + Se treatment plates for another 7 days. **C)** and **D)** Relative root growth between lines grown in control and treatment media. Error bars represent standard error of the mean with n=20.

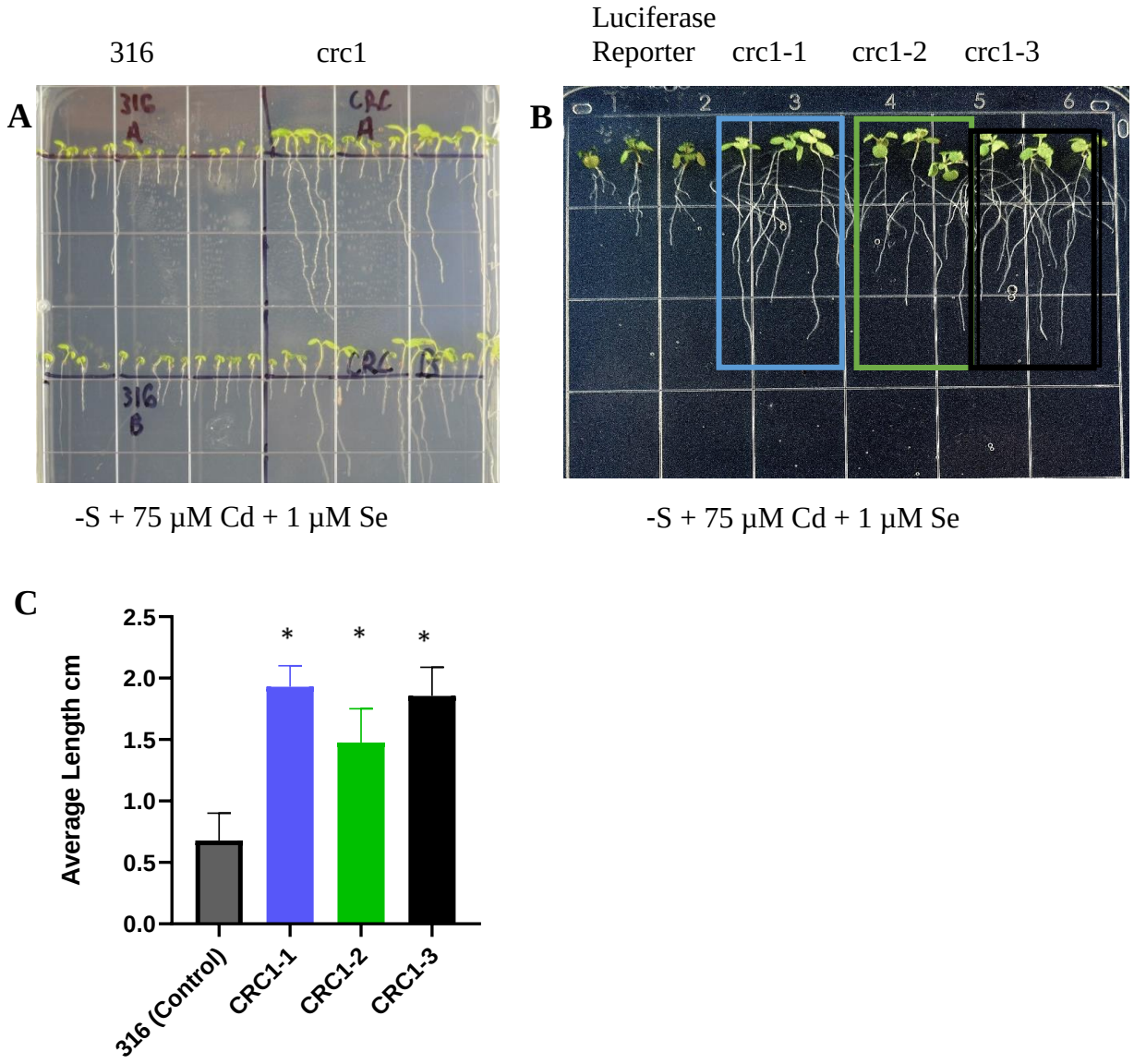

Figure 1.7: Obtaining optimal seed-stock quality. **A)** *crc1* seed stocks lacking the normal *crc1* mutant root-growth phenotype. Mutants showing the phenotype were propagated. Seedlings were allowed to grow in growth chamber conditions for 7 days in -S media before being plated on fresh -S control plates or -S + Cd + Se treatment plates for another 7 days. **B)** Propagated *crc1* mutants showing the normal root-growth phenotype of long roots in -S + Cd + Se media. **C)** Average root- growth of crc1 mutants and the 316 luciferase parental reporter control in -S + Cd+ Se media. N=9, error bars represent standard error of the mean. * indicates $P < 0.05$, one-way ANOVA.

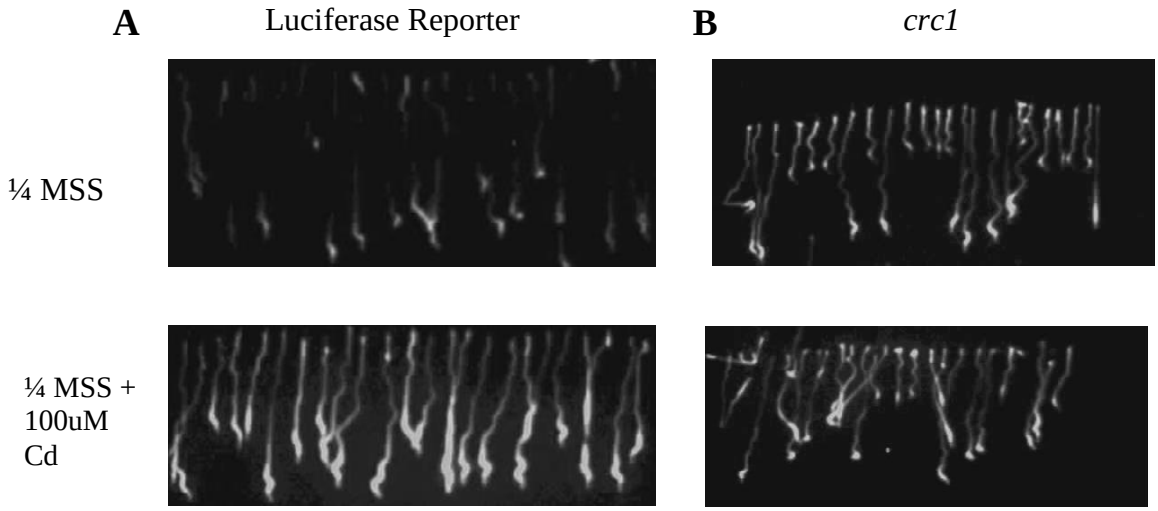

Figure 1.8: Luciferase imaging of propagated crc1 lines and the luciferase reporter. **A)** Propagated luciferase reporter gene in the presence, or absence, of cadmium. **B)** Propagated *crc1* mutants in the presence or absence of cadmium. Handling of all luciferin-containing plates was conducted in dark conditions.

Table 1.1*:* Candidate mutations in *src1*. Candidate region determined by Andrew Cooper.

Position	**Ref**	**Mut**	**Change**	**Gene**	**T-DNA Lines**
7962995	G	A	Ala->Thr	At4g13710	Exon Lines: SALK107508, SALK031335
8231343	G	A	Exon 1 no aa change	At4g14300	Exon Line: SALK005747
8319211	G	A	Intergenic		
8323373	G	A	Intergenic		
8463122	G	A	Exon 4 no aa change	At4g14746	Exon Line: SALK131828
8684896	G	A	Asp->Asn	At4g15230	Exon Line: GK-396605-018295
8735237	G	A	Intergenic		Exon Lines: GK-047B01-016076 SALK023008, SALK023255
8905550	G	A	Transposon	At4g15590	
8987679	G	A	Intron	At4g16215	Intron Line: SALK007385
9179854	G	A	UTR?		Intron Line: SALK007685
9205252	G	A	Ser->Thr Ser->Asn	At4g16267 At4g16270	Intron Lines: SALK021343 (E), SALK061827
9380190	G	A	Exon no aa change	At4g16660	Exon Lines: SALK040786, SALK118733

Table 1.1 Continued

Position	Ref	Mut	Change	Gene	T-DNA Lines
9438789	C	T	Intergenic		SALK_070217
9438792	C	T			
9468488	G	A	Arg->Gln	At4g16820	Intron Line: SALK013856
9497317	G	A	UTR		Exon Line: SAIL559B03
9867766	C	T	Asp->Asn	At4g17440	Exon Line: SALK070529
9982340	G	A	Intergenic		Exon Line: SALK012841
10320392	C	T	STOP	At4g18800	Intron Lines: SALK088879, SAIL868G12
10928676	G	A	Intergenic		Exon Lines: SALK035057 SALK141559 Intron Lines: SALK064931, SALK204731
11005104	C	T	Pro->Leu	At4g20380	Intron Lines: SALK042687, SALK074695
11035408	C	T	Pro->Leu	At4g20480	Exon Lines: SALK039521, SALK097942
11291869	G	A	Ser->Asn	At4g21180	Exon Line: SALK007095
11367850	G	A	Transposon		

Table 1.2*:* Candidate mutations in *crc1*. Candidate region determined by Andrew Cooper.

Position	**Ref**	**Mut**	**Change**	**Gene**	**T-DNA Lines**
6022576	G	A	Exon 2 no aa change	At4g09510	SALK_088756
6265353	G	A	Exon 2 no aa change	At4g10010	SALK_034138 SALK_205532 SALK_113705 SALK_055527
6285157	C	T	Intron 9	At4g10050	SALK_093391 SALK_079539 SALK_064821
6548119	C	T	Exon G -> S	At4g10600	SALK_073717 SALK_113511
6705414	G	A	Intron 6	At4g10930	SALK_067394 SALK_120184C
6936438	C	T	Upstream 2.2 kb	At4g11400	SALK_016155C SK24175
6984153	G	A	Exon 1 no aa change	At4g11510	SALK_112439
7057547	C	T	Exon 1 no aa change	At4g11690	SALK_025470C SALK_136836 SALK_128441
7069256	G	A	Exon 4 G -> S	At4g11730	SALK_019224 SALK_058103
7558982	G	A	Retrotransposon	At4g12915	SAIL_752_C02 SALK_067308

Table 1.2 Continued

Position	**Ref**	**Mut**	**Change**	**Gene**	**T-DNA Lines**
7600257	C	T	Upstream 1 kb	At4g13000	SALK_102683 SALK_101874
			Upstream 300 bp	At4g13010	SALK_109392 SALK_096164
7718518	C	T	10 kb of nothing		
7753939	C	T	Exon no aa change	At4g13320	
7892169	C	T	Downstream 200 bp	At4g13575	SALK_117073 SALK_117071

Table 1.3: Primers used for genotyping and RT-PCR, designed by Andrew Cooper.

Primer Sequence	Gene	Function
TTGATTTAAACACTCGTCCGG	At2g44940	Genotyping SALK_020979C
CTAAGCTTTGTGACGACCCAG	At2g44940	Genotyping SALK_020979C
TTATCGATAACCGGTTTGTGC	At3g60490	Genotyping SALK_111486C
TAAAACAATCCAGACCCATGC	At3g60490	Genotyping SALK_111486C
TAAGGCACGCTTTCTTCTCTG	At2g21045	Genotyping SAIL_816_G07
TTACCGTGAAGGCTTGTAACG	At2g21045	Genotyping SAIL_816_G07
AACATAACACGCGCTTTCAAC	At4g13710	Genotyping SALK_107508
GGAAGAAAACGCCGTTTAAAC	At4g13710	Genotyping SALK_107508
GACACGTGGTCACATCACAAC	At4g13710	Genotyping SALK_031335
GCGTCGTCTTCTCAGAAACTG	At4g13710	Genotyping SALK_031335
GAGCTGTAAACGAAGTGCACC	At4g16267	Genotyping SALK_021343
AATTGGGTTGTTGAAGTGCAC	At4g16267	Genotyping SALK_021343
GAGCTGTAAACGAAGTGCACC	At4g16267	Genotyping SALK_061827
TTCCTTATGTTTTTCTTTGCAATG	At4g16267	Genotyping SALK_061827
TCGTCTAACGGATCCAACAAC	At4g16820	Genotyping SALK_013856
TAAGACAATCCGACCATCCTG	At4g16820	Genotyping SALK_013856
GTGGAGGTTCTTTCCCAGAAG	At4g17440	Genotyping SALK_070529
GCTTGCTCTTGGTGATTTTTG	At4g17440	Genotyping SALK_070529
GCAACCAGTTTTCTTCACTGC	At4g18800	Genotyping SALK_088879
CGAGTCTAAATCAACGATCGG	At4g18800	Genotyping SALK_088879
AAACGGTGGAGACCAGATACC	At4g18800	Genotyping SAIL_868_G12
CTTTCTTGACCAGCAGTGTCC	At4g18800	Genotyping SAIL_868_G12
CTGGGATTTGTAAAGCAGCTG	At4g20380	Genotyping SALK_042687
TCAAGTTCCATGGAGCAAAAG	At4g20380	Genotyping SALK_042687
CATTACCAGGGTCTTTTCGTG	At4g20380	Genotyping SALK_074695
TCGGAAGAAGAGTAATTTGCG	At4g20380	Genotyping SALK_074695
GAATAAAAAGCTGGGGTTTGC	At4g20480	Genotyping SALK_039521
ACCGGAAACGATTTTATGTCC	At4g20480	Genotyping SALK_039521
AATTAGAATTCCTTCGACGGC	At4g20480	Genotyping SALK_097942
CTTTGACTCTTGCAATCAGCC	At4g20480	Genotyping SALK_097942
AATCTCTGCGTATTCAGCTGC	At4g20480	Genotyping SALK_007095
GAGTGTGATCGTTCAGGGAAG	At4g20480	Genotyping SALK_007095
GAAGGCACTCAAGGCCTCAT	At4g16860	Genotyping SAIL_559_B03
AACTTTTGCACCCGTTGAGA	At4g16860	Genotyping SAIL_559_B03
AACGGTTCTCGAACCAATAGG	At4g17980	Genotyping SALK_012841
TTGGTCCAATTAATGATTGAGAAG	At4g17980	Genotyping SALK_012841
ACTTTTGTTGTCAACATCGGC	At4g16770	Genotyping SALK_070217

Table 1.3 Continued

Primer Sequence	Gene	Function
ATTTTGCCGATTTCGGAAC	N/A	Genotyping SALK lines (LBb1.3)
TAGCATCTGAATTTCATAACCAATCTCGATACAC	N/A	Genotyping SAIL lines (LB3)
AGAGTTGAACGAGATTGCAGC	At4g09510	Genotyping SALK_088756
TGGTAAGGAAAAATGTCGACG	At4g09510	Genotyping SALK_088756
TATTGCTTTGCCTCCAAAGTC	At4g10010	Genotyping SALK_034138
CTCTAGCCATGAATCTCACGC	At4g10010	Genotyping SALK_034138
AGAATGCAACCAACACTCCAC	At4g10010	Genotyping SALK_205532C
TTTAGCACATAAACCATCCGC	At4g10010	Genotyping SALK_205532C
TTTTCCGTTATCGACGAACAG	At4g10010	Genotyping SALK_113705
CTCTAGCCATGAATCTCACGC	At4g10010	Genotyping SALK_113705
CTTAGTGTCGAAGAAGCCGTG	At4g10010	Genotyping SALK_055527
TGCAGATATTGGAACAGGAGG	At4g10010	Genotyping SALK_055527
CGAACAAAGCCAACATCTTTC	At4g10050	Genotyping SALK_093391
CAACAGGTCCTTCATTTCCTG	At4g10050	Genotyping SALK_093391
GAGATCGAAAACAGCATACCG	At4g10050	Genotyping SALK_079539C
ATCATATGGGCCTAAAATGGC	At4g10050	Genotyping SALK_079539C
GTGAGGGTCCTAATCCAATCC	At4g10050	Genotyping SALK_064821
ATTAACACACAGCATGGGAGG	At4g10050	Genotyping SALK_064821
TGCATGGGTTATTCAAAGATTC	At4g10600	Genotyping SALK_073717C
CCATGTATGAACCCAAACACC	At4g10600	Genotyping SALK_073717C
CCATACCGGCCCATATTTTAG	At4g10600	Genotyping SALK_113511
CAAATGAAACCATGAACCATTG	At4g10600	Genotyping SALK_113511
GTTCTGTCGCTGCACTTCTTC	At4g10930	Genotyping SALK_067394
GAAGTTGATGCTGCAGAAAGG	At4g10930	Genotyping SALK_067394
GTTCAGTTTTGATCCAGCAGC	At4g10930	Genotyping SALK_120184C
TCAGATGTTGCCAGTGTCATC	At4g10930	Genotyping SALK_120184C
CCTCCCTATCGACTTTCGATC	At4g11400	Genotyping SALK_016155C
CCATGTTTGTTTGTTTGACCC	At4g11400	Genotyping SALK_016155C
TTTACCGGGAATGTTGAAGTG	At4g11400	Genotyping SK24175
AGTCACACGGTTCTGATACCG	At4g11400	Genotyping SK24175
TTTCAGTTTTTGTTTTGTTGGG	At4g11510	Genotyping SALK_112439
GGCATTGGTGTTTCATGTTTG	At4g11510	Genotyping SALK_112439
CTGCATAAACTCAACGCCTTC	At4g11690	Genotyping SALK_025470C
TTTAAACGTATCTGCCATGGC	At4g11690	Genotyping SALK_025470C
TCTCACCTTTCTTGCAACACC	At4g11690	Genotyping SALK_136836
ATTTGGGTGTCAAAGCAATTG	At4g11690	Genotyping SALK_136836
TTTTGTTCCTGGATCGAATTG	At4g11690	Genotyping SALK_128441

Table 1.3 Continued

Primer Sequence	Gene	Function
TCTAATTCCAGCTCTTGCCTG	At4g11730	Genotyping SALK_019224
TTGTTTTTGTGGGTTACAAAATAG	At4g11730	Genotyping SALK_058103
AACCGATATCTTCCCGGTATG	At4g11730	Genotyping SALK_058103
CATCCTTTTTGTTAGGTGATTGG	At4g12915	Genotyping SAIL_752_C02
CCATCCATGTTGAAATCCATC	At4g12915	Genotyping SAIL_752_C02
TTGTGCATAGGAGTGTTGCAG	At4g12915	Genotyping SALK_067308
ACCAAAAGTTGTTTCGCATTG	At4g12915	Genotyping SALK_067308
TCGTTGCTCTGGGAATCTATG	At4g13010	Genotyping SALK_109392C
TGACAAACTCTATGTTCCGGG	At4g13010	Genotyping SALK_109392C
GCTACAACTTTGTCACCAGCC	At4g13010	Genotyping SALK_096164C
GAAAACTCATGCACGCTCTTC	At4g13010	Genotyping SALK_096164C
CCACAATCTTCTTTTTCGTCG	At4g13000	Genotyping SALK_102683
GGTCCATTATCAAACTCCGAG	At4g13000	Genotyping SALK_102683
CGACTCTCAACTTCGACCATC	At4g13000	Genotyping SALK_101874C
AATGGCCAGTTTGACAATTACC	At4g13000	Genotyping SALK_101874C
TGGGAAGGATAAAACGATACG	At4g13575	Genotyping SALK_117071/SALK_117073
CCCATGCAACAAATTAACGAC	At4g13575	Genotyping SALK_117071/SALK_117073
CAGACCACCATCCACGACTT	TaPCS1	qPCR
ACAGCCTGTTCATTCCCTTT	TaPCS1	qPCR
TTAAAGCTGGAGAAAGTATACCGA	HMA3s	qPCR
GCTAGAGCTGTAGTTTTCACCT	HMA3s	qPCR
TGTAACGCTGGAGGAAGAG	At1g79000 (AtHAC1)	qPCR
CTTGCAAGCAATCTTGAGG	At1g79000 (AtHAC1)	qPCR
TGACCTTGAGGCGTACTTGCAC	At4g16820.1	qPCR
TGCCCGAAACGGACAGTTAGATG	At4g16820.1	qPCR
TCGGCGACCTTATGGAAAGATGG	At4g16770.1	qPCR
ACCACTCTATGCAATGTTGATCG	At4g16770.1	qPCR
GGCTATGGTGGGATTATTGTCCAG	At4g16860.1	qPCR
TTGAGGCTTCCAAGTGGCTGAG	At4g16860.1	qPCR
TGGGAGTTGCCAGGTAAATCTTTC	At4g17980.1	qPCR
TTCCGGTCTTTACCAGTTGCTTTC	At4g17980.1	qPCR
ACACTGCTGGTCAAGAAAGATACC	At4g18800.1	qPCR
CGAATGTTGAGTGTCGGGTTACG	At4g18800.1	qPCR
TGACTCTTGCAATCAGCCTCTGG	At4g20480.1	qPCR
TCCTCAAGAGCTACTGGATCTGC	At4g20480.1	qPCR
CAGAATCTTGGCCTTTCACAAACC	At4g16270.1	qPCR
TCCCAATGTATGTCCACCGGAAAG	At4g16270.1	qPCR

Table 1.2 Continued

Primer Sequence	Gene	Function
TGACCTGTTCCCACTCATGCTG	At4g174402	qPCR
TGAAAGCGGAAAGTTGGTGAGC	At4g203801	qPCR
TGCACAAACCAAACGCAGGAATAG	At4g203801	qPCR
AGAACATGAGCCGTGAGAGTCAAC	At4g211801	qPCR
TCTGAAGCACCAGGTTCCAAAC	At4g211801	qPCR
CAACAATGGCGGCGATAAGGAAG	At4g137101	qPCR
AACGGCGTGTTGTTGTTGTTGTG	At4g137101	qPCR
TGTAACAAGATGGATGCCACCAC	At5g60390 (EF1α)	qPCR
TCCCTCGAATCCAGAGATTGGC	At5g60390 (EF1α)	qPCR
GCCTGTATTGGAGCATTCTTTGGC	At1g78000.1 (SULTR1;2)	qPCR
ATCTTAGCAAACGAGATCGAGACG	At1g78000.1 (SULTR1;2)	qPCR
GCTGGAACTGATAGGTTGGACAGG	At4g10050.1	qPCR
TCCGGTACATCTTCCTGTATGGC	At4g10050.1	qPCR
AGCTGCAGTGTCAACCGATACC	At4g12915.1	qPCR
TTTGGCTCTCAGCTGCTCGATG	At4g12915.1	qPCR
TGGATATCTGCTTGAACGAAACGG	At4g13575.1	qPCR
TCACCCGACTTAACCTCACGTTG	At4g13575.1	qPCR
TTTCCTCACACGGCTCATTGCC	At4g14030.1	qPCR
TGTCCTGGTTTCTCCCACCTATTC	At4g14030.1	qPCR
CTGTCCCGTTCGCAAACAAGTTC	At1g62300.1 (WRKY6)	qPCR
CGGCAACGGATGGTTATGGTTTC	At1g62300.1 (WRKY6)	qPCR

1.5: Discussion

Super Response to Cadmium (src1)

From the RT-PCR experiments conducted on the T-DNA knockout lines for candidate genes responsible for the *src1* luciferase phenotype, At4g15230 and At4g16267 knockouts have greater *SULTR1;2* expression in response to cadmium, and mirror the response of the *src1* mutant (figure 2). While these results are interesting, it is important to note that the *SULTR1;2* expression of all candidate genes must be evaluated (Table 1). After this has been completed, all candidate genes displaying *SULTR1;2* expression mirroring that of the *src1* mutants will subsequently have a qualitative polymerase chain reaction (qPCR) conducted in order to provide data that is more easily quantifiable. In addition, qPCR experiments will be performed on the luciferase reporter and *src1* lines. Once discovered, genes displaying *SULTR1;2* expression most similar to that of *src1*, in response to cadmium, will then be then be backcrossed to Columbia 0 wildtype Arabidopsis to test for complementation. Simultaneously, T-DNA candidate lines will be backcrossed with the *src1* mutant to test for allelism of the candidate gene(s) in the F1 generation. Once these experiments have been successfully conducted, the gene(s) determined to be responsible for the phenotype of the *src1* mutants will be knocked out via CRISPER-CAS9 mutagenesis in the original luciferase reporter. Upon the obtainment of successful knockouts of these genes, gene expression of *SULTR1;2* will be obtained by qPCR and compared to that of the *src1* mutant population. For all necessary RT-PCR, qPCR, and PCR reactions needed for future experiments, primers have been already procured (Table 2). All *src1* T-DNA lines have been bulked and genotyped for knockout homozygosity. At4g15230 encodes an ATP-binding cassette

transporter that has been implicated in epidermal root transport of specific carbohydrates, and has been displayed to effect the number of soil bacteria surrounding the plant, especially those pertaining to heavy metal remediation (Sugiyama et al., 2009). However, the exact function of the encoded protein is not known. In Sugiyama et al, the authors investigate whether or not this gene has direct impact on microbial communities, or if At4g15230 is encoding for a protein that is enacting some effect on the plant. Since most of our assays are conducted with sterilized seeds on sterile media, our research would suggest the latter. At4g16267 encodes for a plant thionin defense protein, which are composed of sulfur-rich peptides (Silverstein et al., 2007). Since the super response to cadmium of the *src1* mutants is determined by *SULTR1;2* expression, it is encouraging that both candidate genes have been implicated with heavy metals or sulfur.

To determine if the *src1* mutants are accumulating different amounts of cadmium and selenium relative to the reporter control, Inductively Coupled Plasma-Optical Electron Spectroscopy (ICP-OES) will be conducted. In addition, knockout mutants of the gene(s) thought responsible for the *src1* phenotype will also undergo ICP-OES analysis to see if cadmium and selenium accumulation mirror that of the *src1* mutant.

Constitutive Response to Cadmium (crc1)

Originally the *crc1* seed stock was not of optimal condition, causing forward experiments to be quite difficult to perform. After propagating *crc1* mutants displaying the -S + Cd + Se root-growth phenotype, luciferase assays were performed to ensure the presence of the SULTR1;2 reporter gene construct. In addition, many of the T-DNA knockout lines were dwindling in number. These lines were replenished and can be implemented in -S +Cd +Se root-growth assays

to duplicate Andrew Cooper's findings. Once candidate genes can be established, they can be tested for allelism and complementation against the *crc1* mutants by means of cross-pollination. Simultaneously, CRIPSR-CAS9 mutants of the candidate genes can be constructed. Then, these lines would be tested in the -S +Cd +Se root-growth assays to ensure that root-length would be comparable to that of the original *crc1* mutants. The two genes currently thought to be responsible for the constitutive response to cadmium of the *crc1* mutants are At4g10930 and At4g13575. At4g10930 encodes for a U-BOX protein, which has been implicated in protein degradation in plants (Andersen et al., 2004). It would be interesting to investigate if additional U-BOX protein knockouts would have similar effects on plant signaling processed in response to cadmium. Interestingly, At4g13575 encodes for a hypothetical protein. Localization experiments would be particularly interesting to determine where this protein was accumulating in Arabidopsis, in attempt to elucidate the function of this hypothetical protein.

To determine if the *crc1* mutants are accumulating different amounts of cadmium and selenium relative to the reporter control, Inductively Coupled Plasma-Optical Electron Spectroscopy (ICP-OES) will be conducted. In addition, knockout mutants of the gene(s) thought responsible for the *crc1* phenotype will also undergo ICP-OES analysis to see if cadmium and selenium accumulation mirror that of the *crc1* mutant.

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II.

Monitering Heavy Metals and Metalliods in Crops Produced from Campus Community Gardens

2.1 Abstract:

Throughout the United States, many communities do not have affordable access to quality food products. To ameliorate this problem, many communities have pooled together resources for the construction of community gardens. In addition to serving as a place to grow fresh produce, these community gardens have been shown to increase constructive social interactions between members of the community. However, years of prior industrial activity have deposited harmful amounts of heavy metals and metalliods into the native soils of some of these community gardens. There is an increasing concern that harmful levels of heavy metals and metalliods may be accumulating in the edible plant tissues of these urban community gardens.

ICP-OES analysis was performed by our laboratory on plant tissues from the Ocean-View Growing Grounds (OVGG), an urban community garden in Southeast San Diego. These analysis displayed potentially harmful levels of heavy metals in edible plant tissues, which were ameliorated with the introduction of raised growing beds. A majority of the land that comprises the University of California, San Diego campus was at one time a military base. Like many urban soils, native soils of military bases have also shown elevated levels of heavy metals and metaloids. Due to this potential concern, ICP-OES analysis was performed on plant samples from two major community gardens within the campus of UCSD, to determine if potentially harmful levels of heavy metals and metalloids could be detected. To date, large concentrations of heavy metals in tissues from these campus community gardens have not been detected in edible tisues of produce.

2.2 Introduction:

Due to lack of reliable transportation, distance to supermarkets offering affordable produce, and income inequality, “food deserts” are arising throughout the United States and are characterized as being communities that do not have available produce for the resident populace (USDA 2008). Indeed, low-income urban food deserts struggle to provide high-quality produce that is cruicial for adequate nutrition of the residing populace (Hendrickson, Smith, & Eikenberry, 2006). For many of the people residing in food desserts, vegetables and produce are highly desired, but are not purchased. Instead, residents often purchase processed foods high in starch for reasons of practicality and affordabilitiy (Koh & Caples, 1979). Inadequate consumption of fruits and vegetables has directly been linked to a plethora of diseases, including diabetes, cancer, coronary heart disease, pulmonary diseases, and even cataract formation (Van Duyn & Pivonka, 2000; Who & Consultation, 2003). Due to the large consumption of starchy processed foods in lieu of expensive vegetables and produce, as well as other sociological factors, many of these urban food deserts have higer rates of obesity (Macintyre & Cummins, 2005). To add additional stress, urbanization across the world is increasing at an alarming rate, and can be expected to further exasterbate this problem of nutritional insecurity (Starke, 2007).

To allieviate the burden of food insecurity faced in these urban food deserts, many cities globally have employed considerable efforts in the develpoment of urban community gardens. Urban community gardens are vacant lots of land in urban areas where social and financial capital is pooled together to create an area condusive to crop cultivation (Glover, Parry, & Shinew, 2018). In addition to this, community gardens often serve as areas of community social engagement, and recreation (Guitart, Pickering, & Byrne, 2012). A study in Flint, Michigan, showed that active participants of community gardens have been shown to use community

gardens for the cultivation of crops, and consume fresh produce on an average of almost twice that of non-participating members of the community (Alaimo, Packnett, Miles, & Kruger, 2008). In this urban food desert almost 15% of residents either themselves participated in a community garden, or had a family member that did, suggesting that community gardens had a positive impact on the nutritional obtainment of populaces living in urban food deserts.

While urban community gardens have many beneficial aspects, it must be remembered that many of these urban soils have been involved in years of accumulated industrial activity, resulting in potential heavy-metal and metalloid contamination (Alloway, 2013). Due to this, several studies have emerged with the purpose of elucidating the potential risk these soils may have on people involved with urban community gardens, and have shown harmful levels of heavy metals and metalliods in soils (Marquez-Bravo et al., 2014; Taylor et al., 2013). Many of these heavy metals and metaliods, such as lead, cadmium, arsenic, mercury, and nickle, can cause a plethora of health effects, and have been implicated in the formation of many cancers and damage to organ systems in humans (Aoshima, 2012; Cao et al., 2014; Lane, Canty, & More, 2015).

This current research involves itsself with the Ocean-View Growing grounds (OVGG), an urban community garden located in Southeastern San Diego, as well as several community gardens located around the campus of the University of California, San Diego. Due to the possiblity of urban soil being contaminated at the, Andrew Cooper previously measured the heavy metal content of several fruit and vegetable varieties at OVGG, and found potentially harful levels of Arsenic in several edible plant tissues (Cooper, 2018). However, the implication of raised beds with imported soil prevented new plants from obtaining arsenic contamination.

Originally the area of land that now consists of the campus of the University of California, San Diego, served as a military (marine) base. Such establishments historically have shown elevated levels of heavy metals in the soil due to the use of munitions (Roman G. Kuperman; Margaret M. Carreiro, 1996). In order to ensure that crops grown on campus community gardens are deviod of heavy metal contamination, Inductively-Coupled Plama Optical Emission Spectroscopy (ICP-OES) on various plant tissues from three campus community gardens, was persued.

2.3. Materials and Methods

ICP-OES Analysis

First, solutions of 1000, 500, 250, 125, and 62.5 ppb were created using Clarits PPT Multi-Element Solution 2, and served at our standard for ICP-OES heavy metal and metalliod analysis. For tissue harvesting, leaves and fruit were picked from three community gardens across the UCSD campus, and were stored at 60 degress celcius for 5 days to dry. For each plant sample harvested, n = 3. To prepare the samples for ICP-OES analysis, they were first digested with 68% nitric acid for 3 days, and then boiled for 3 hours. After digestion, all samples were diluted 20 times using distilled MicroPore water. Finally, samples were processed with a Perkin Elmer Optima 3000 DV ICP-OES in the lab of Dr. Castillo at Scripps Institution of Oceanography.

2.4: Results

Previously, Andrew Cooper performed ICP-OES anlaysis on plant samples from the Ocean-View Growing Grounds (OVGG) and was able to find potentially harmful levels of arsenic in edible tissues (Figure 2.1A). Notably, the subsequent employment of raised growing beds with imported soils prevented similar levels to be remeasured for future plants of the exact species (Figure 2.1B).

The presence of heavy metals and metalliods in plant tissues at the OVGG prompted us to determine if the same would hold true of plants at Elie's and Roger's Campus Community gardens on the UCSD campus. To determine if plants from campus community gardens were absorbing dangerous levels of heavy metals and metalliods, ICP-OES was also performed on samples collected from Roger's Garden and Ellie's Garden, two of the more prominent campus community gardens at UCSD (Figure 2.2A, Figure 2.2B). Interestingly, Ellie's community garden showed elevated cobalt levels, with the highest reading of 5ppm cobalt coming from argo mustard seeds (Figure 2.2B). Neither of the community gardens displayed heavy metal or metalloid contamination in edible fruit tissues. In Ellie's Community Garden, lime tree leaf samples displayed an arsenic concentration of about 1 ppm (figure 2.2A).

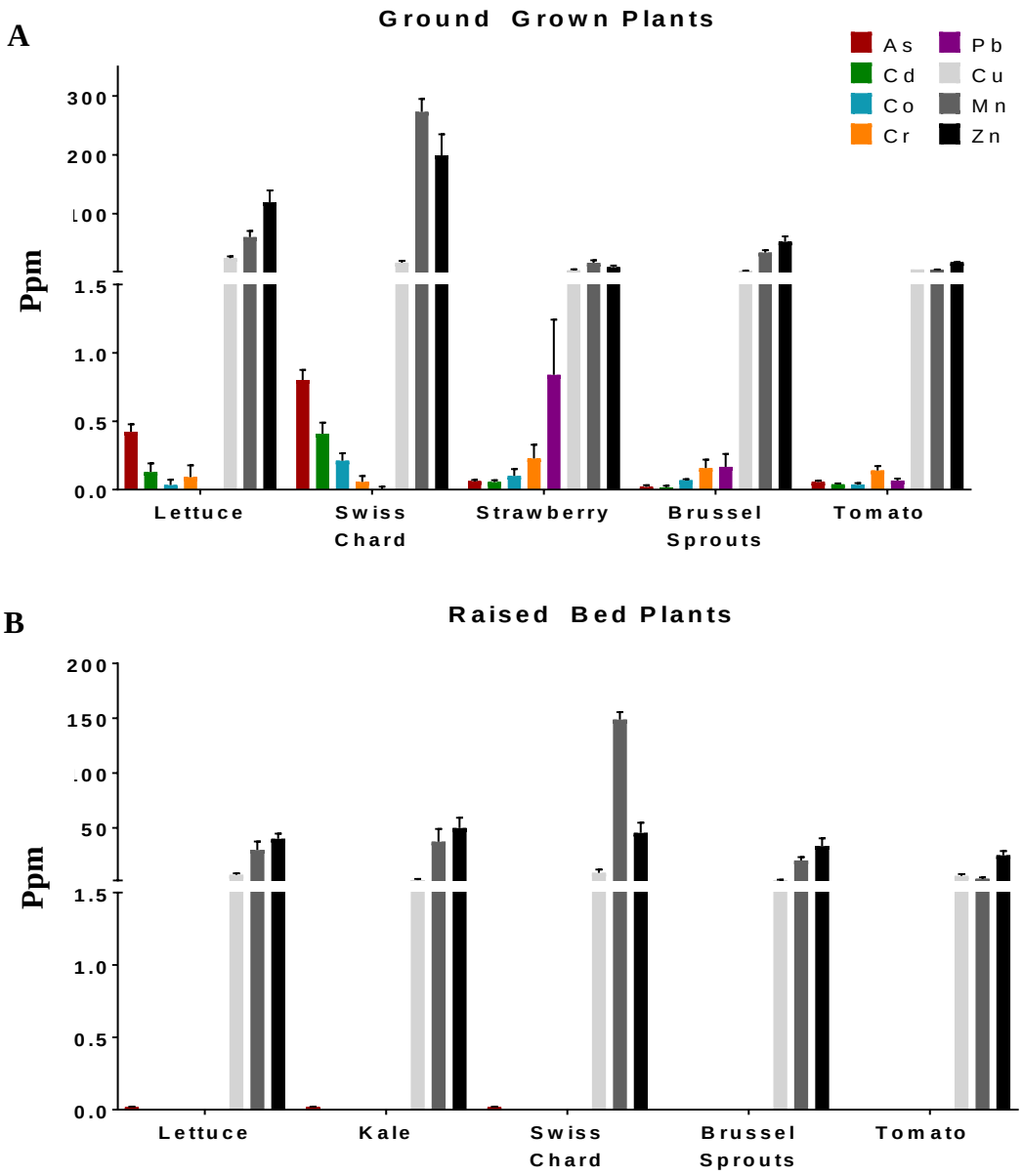

Figure 2.1: ICP-OES analysis of produce from the OVGG in Southeastern San Diego. Performed by Andrew Cooper. Error bars represent standard errors from the mean. **A)** ICP-OES analysis of plant samples grown in the native soil of the OVGG. **B)** ICP-OES analysis of plant samples grown in soil imported for the contruction of raised beds. Source: Andrew Cooper.

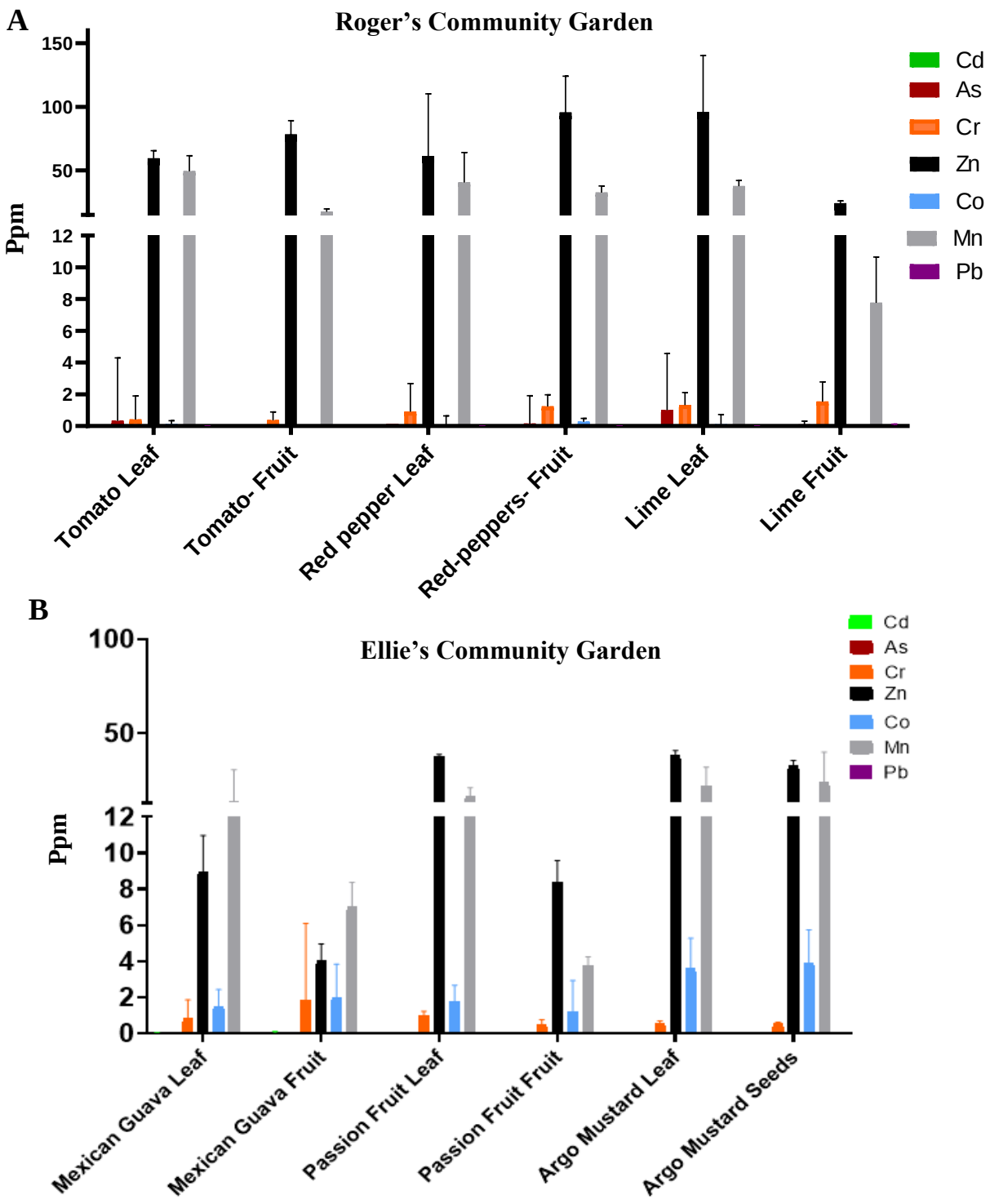

Figure 2.2: ICP-OES analysis performed on plant samples from communty gardens around the campus of the University of California, San Diego. Error bars represent the standard error from the mean. **A)** ICP-OES analysis of Roger's Community Garden. **B)** ICP-OES analysis of Ellie's Community Garden

2.5: Discussion

Currently there are no known guidelines that determine the toxic concentration of heavy metals within produce. However, the Agency for Toxic Substances and Disease Registry (ATSDR) has established regulatory guidelines pertaining to permissible concentrations of heavy metals and metalliods in drinking water, which were determined to be 0.01 ppm for arsenic, 0.005 ppm for cadmium, and 0.015 ppm for lead (ATSDR arsenic, 2007; ATSDR cadmium 2012; ATSDR Lead, 2007).

From these standards listed by ATSDR, many of the samples grown in the native soil of the OVGG contained worrisome levels of heavy metal and metalliod accumulation. The implimentation of raised beds has, in past studies, ameliorated heavy metal concentrations of plants grown in urban soils (Marquez-Bravo et al.), and remain effective for this study.

In past research, the partitioning of heavy metals and metalliods amongst tree tissues was shown to be not equal, as fruits of these trees had significantly less heavy metals and metalliods, suggesting a potential capability of trees to sequester toxic compounds away from sensitive tissues (Wright, R.T., 2007; Li et al., 2006; Sawidis, Chettri, Papaioannou, Zachariadis, & Stratis, 2001; Cooper, 2018). For Roger's community garden, tomato leaf, red pepper fruit, lime leaf, and lime fruit were the only samples that displayed any measurable arsenic presence. Lime leaf samples dislpayed the highest levels of arsenic measured, at a concentration about 1 ppm. However, lime fruit had significanty less arsenic accumulation, again suggesting the potential existance of a mechanism involved in the sequestration of heavy metals and metalloids from the sensitive tissues of trees.

None of the samples in Ellie's Garden displayed detectable heavy metal contamination, but contained elevated levels of cobalt, compared to samples from Roger's community garden. Unlike lead, cadmium, and arsenic, cobalt has important biological implications for the human diet and is a major cofactor in vitamins essential for a functioning metabolism (Wright, R.T., 2007; Marston, 1952). Currently, the ATSDR does not have guidelines of permissibility for cobalt concentrations in drinking water, and emphasizes the importance of cobalt in agriculture throughout the United States (ATSDR cobalt, 2007).

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