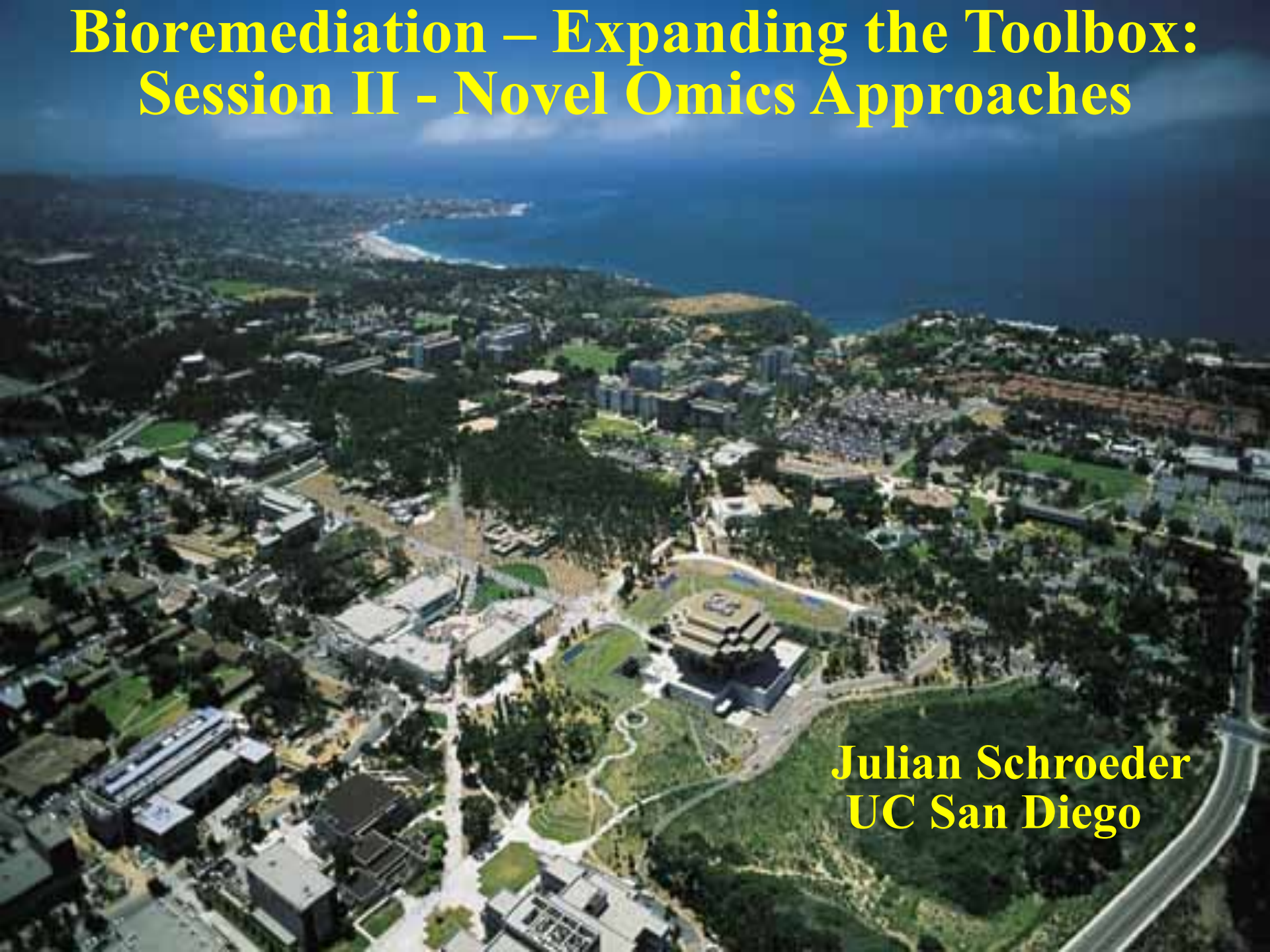
Bioremediation – Expanding the Toolbox: Session II - Novel Omics Approaches

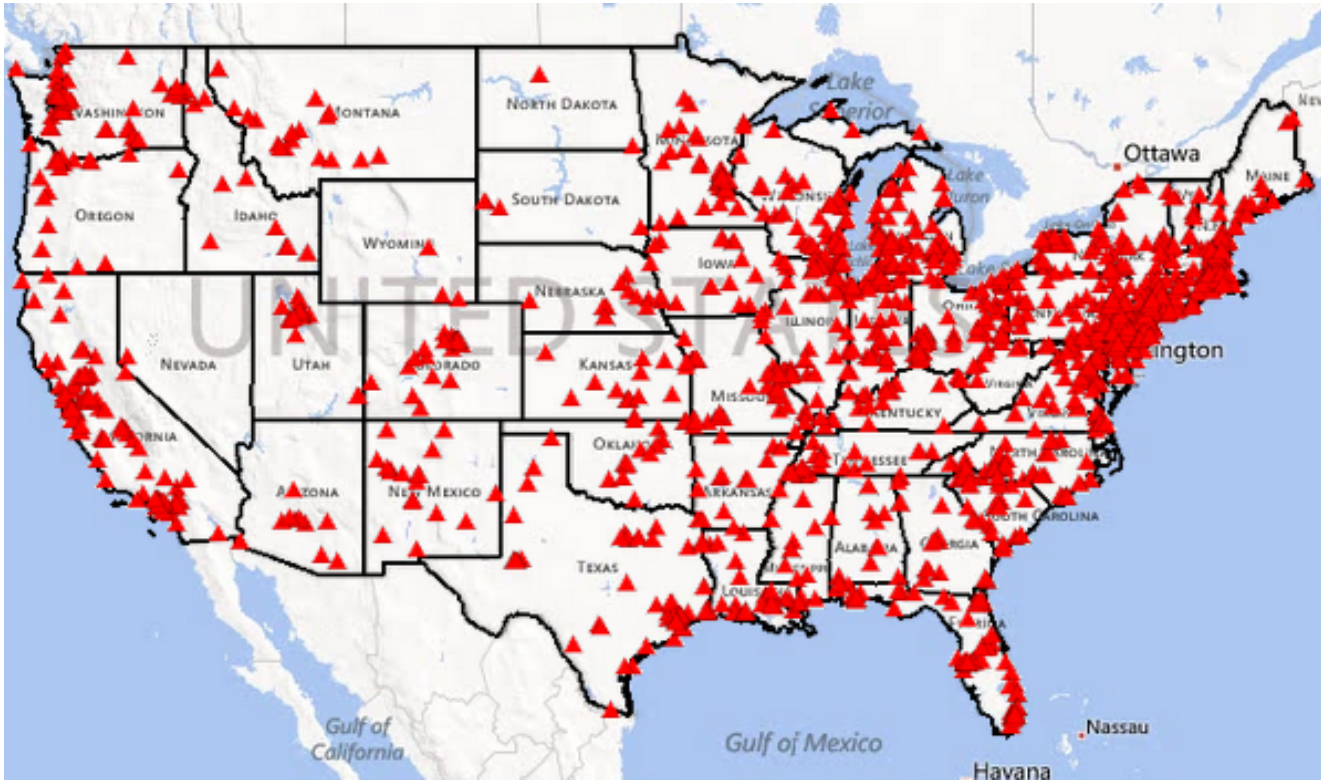


Julian Schroeder
UC San Diego

Some Sources of Heavy Metal and Arsenic Contamination

Highly Contaminated Sites Within The US

> 1000 Superfund sites requiring urgent attention



Priority Substances

1. Arsenic

2. Lead

3. Mercury

4. Vinyl Chloride

5. PCBs

6. Benzene

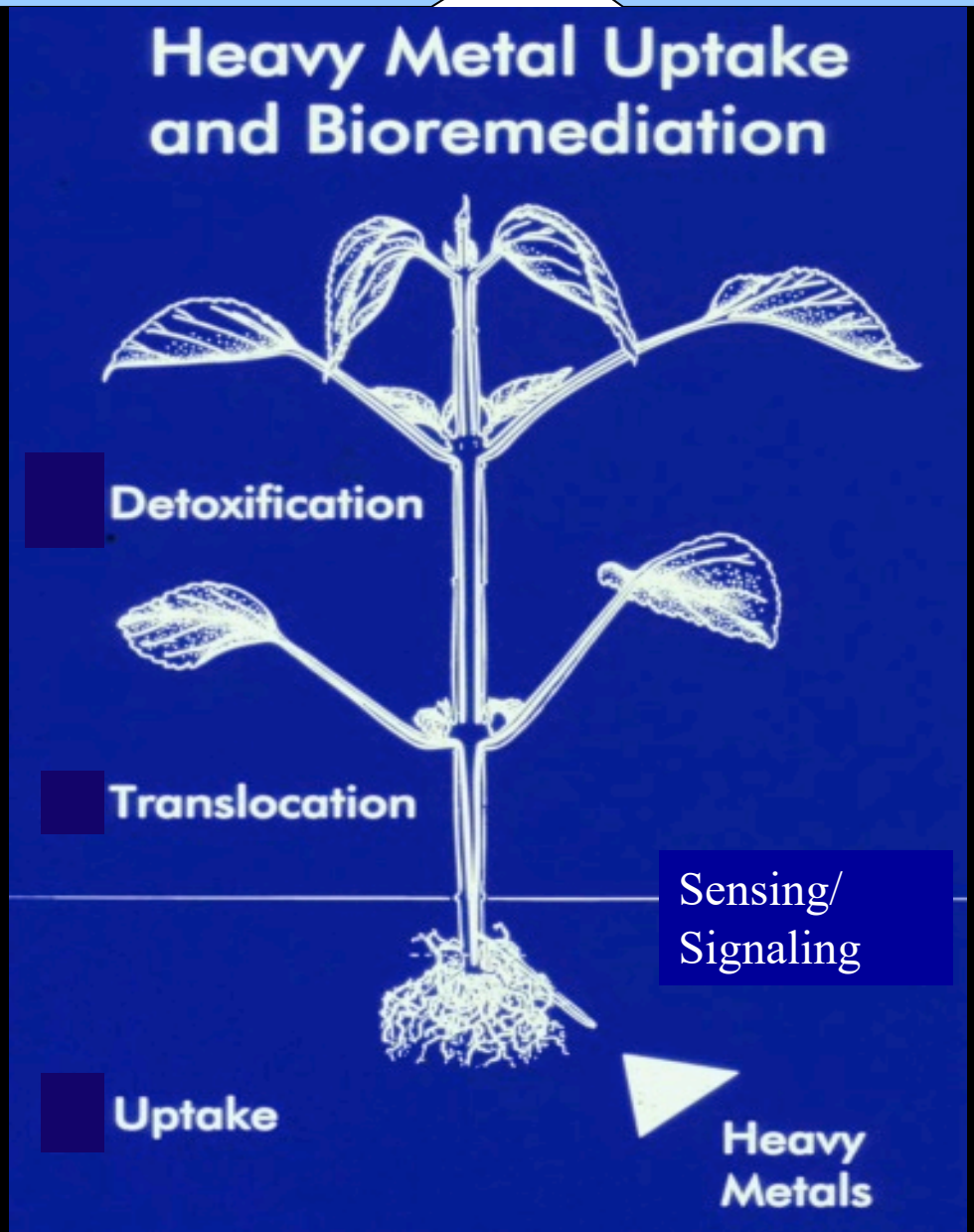
7. Cadmium

8. Benzo(a)pyrene

9. PAHs

Superfund sites in the National Priority List

Agency for Toxic Substances and Disease Registry (www.atsdr.cdc.gov)


Heavy Metal Uptake and Bioremediation
Detoxification
Translocation
Sensing/ Signaling
Uptake
Heavy Metals

cad1-3 is a loss of function mutant in *AtPCS1* and is cadmium sensitive

Control

cad1-3

Wild type (Col0)

cad1-3 is a loss of function mutant in *AtPCS1* and is cadmium sensitive

Control

40 μM Cd^{2+}

cad1-3

Wild type (Col0)

cad1-3

35S:: TaPCS1

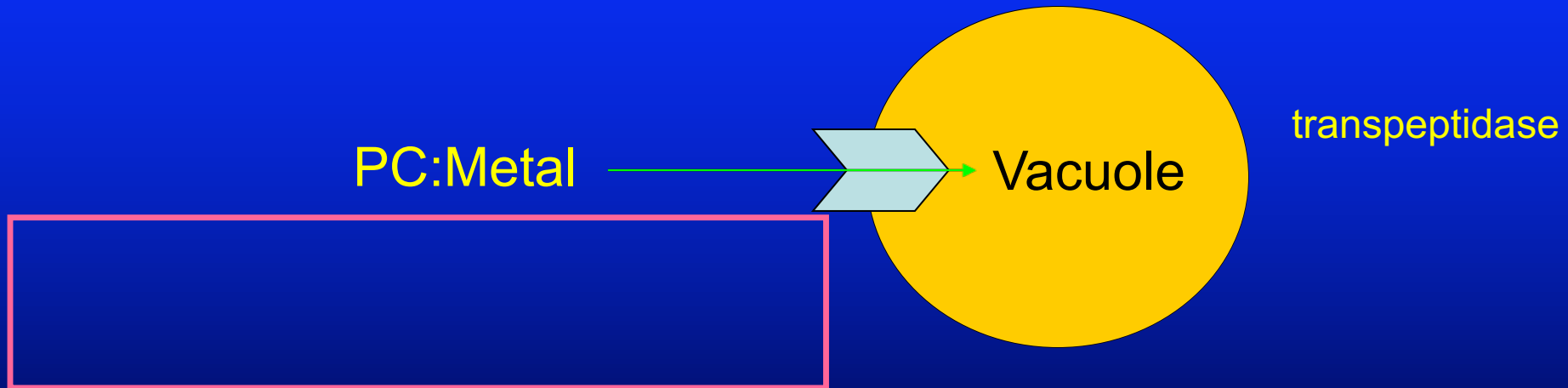
Phytochelatins

Phytochelatins are small metal binding peptides, synthesized in response to the intracellular presence of heavy metals.

Glu + Cys → Glutathione → Phytochelatin

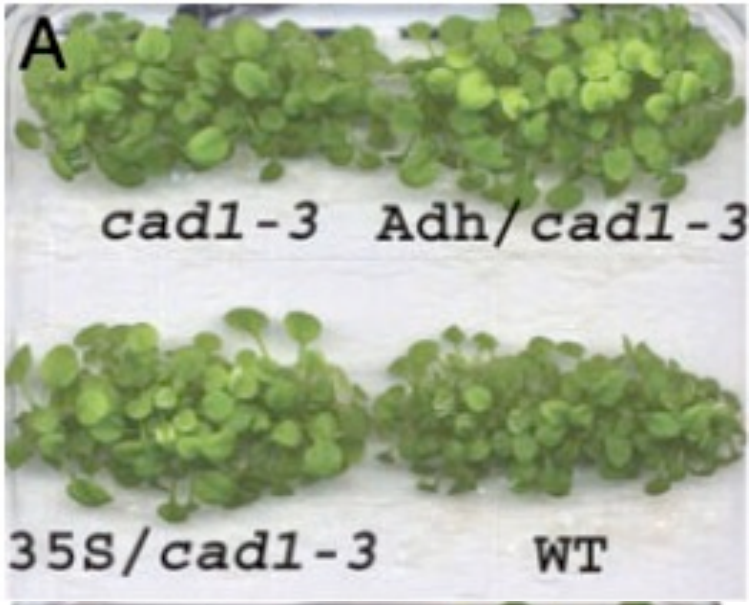
Glutathione: γ-Glu-Cys-Gly

Phytochelatin: $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, n = 2-11

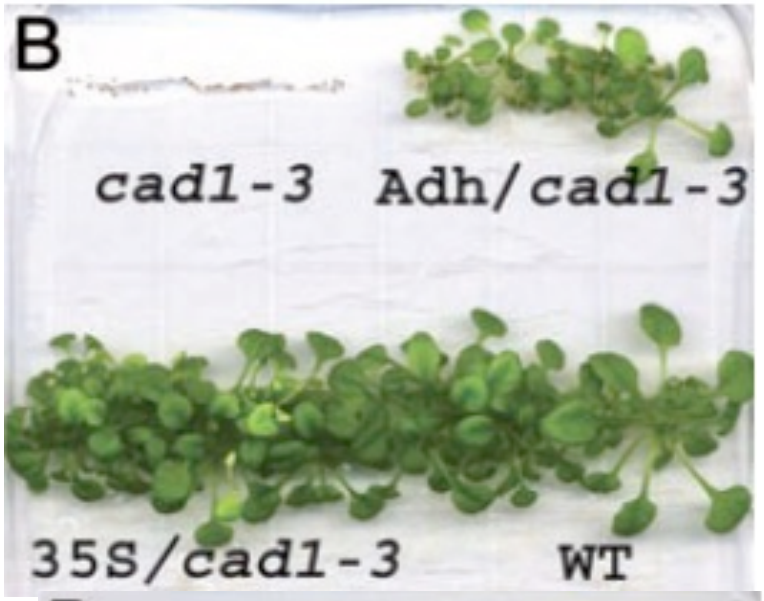


PCS Gene is Essential For Heavy Metal & Arsenic Resistance of Plants

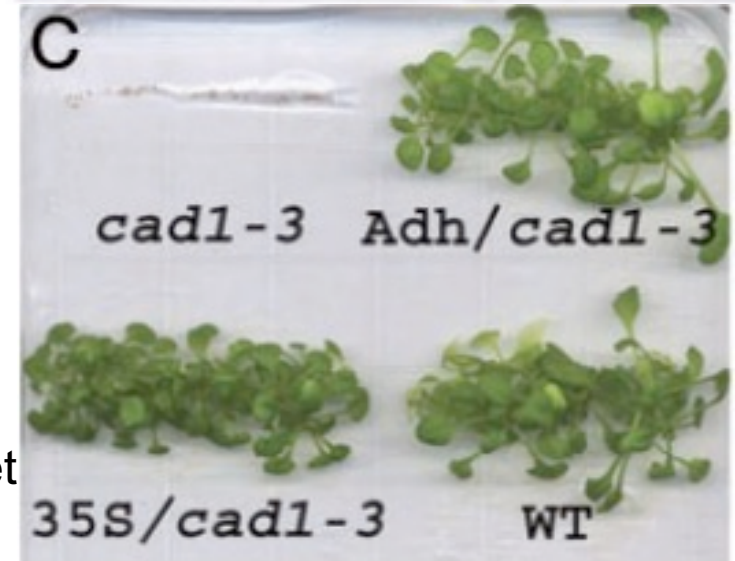
Control



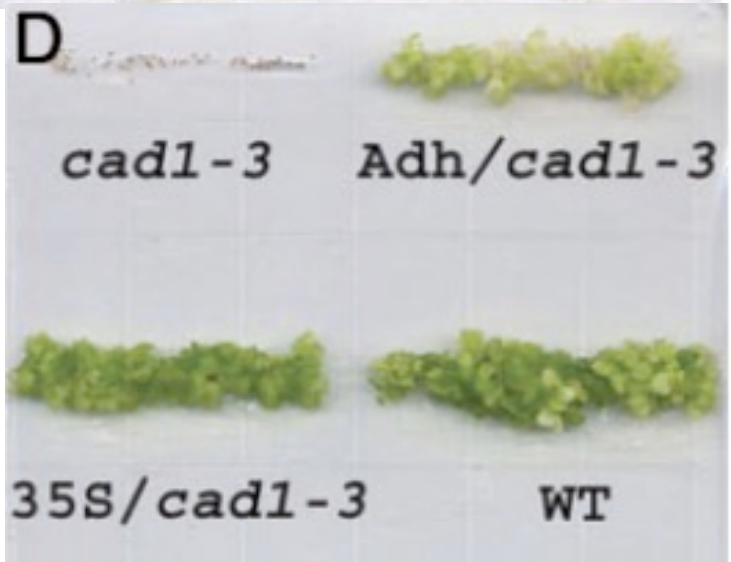
80 μM As



10 μM Hg

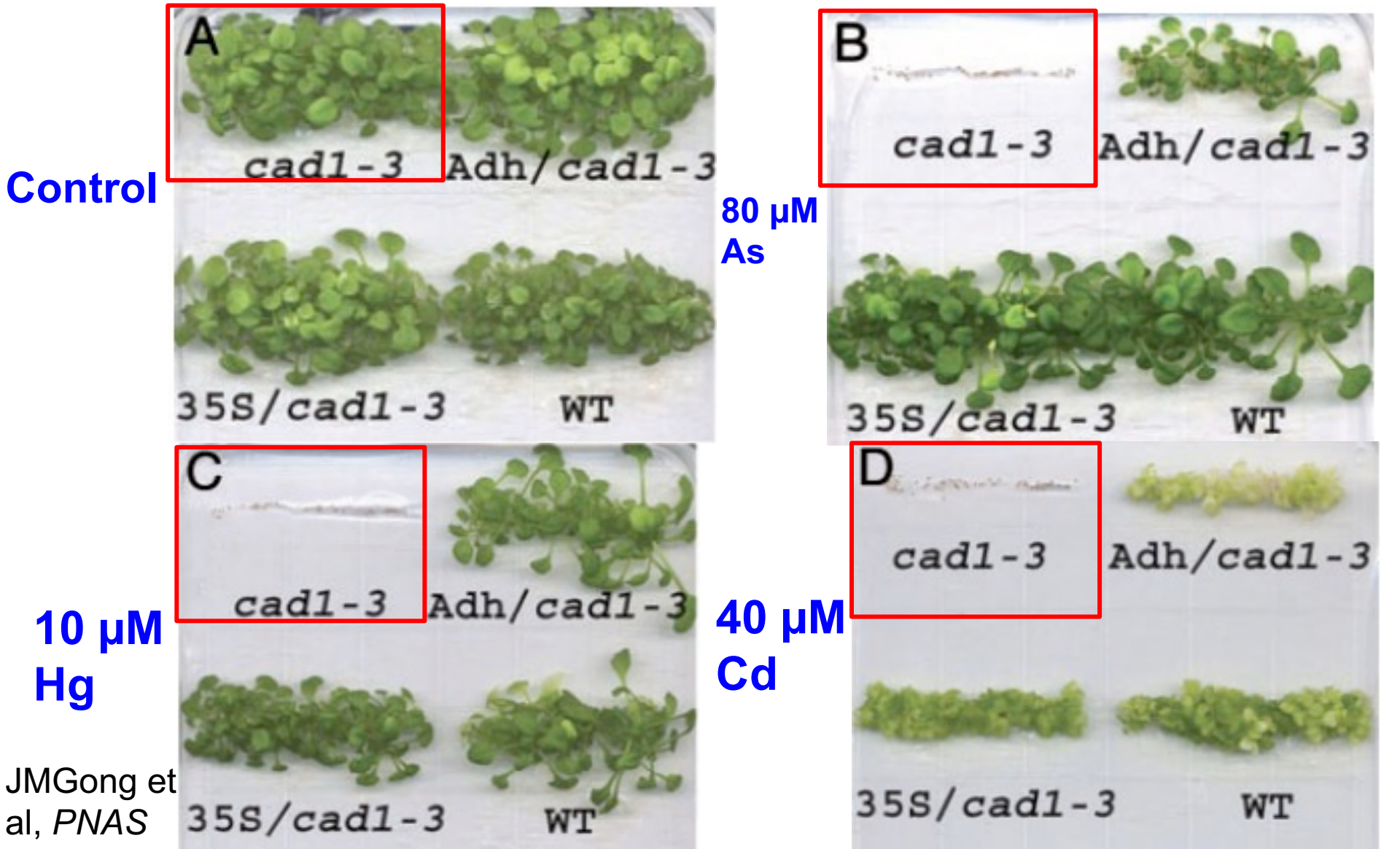


40 μM Cd



JMGong et al, *PNAS*

PCS Gene is Essential For Heavy Metal & Arsenic Resistance of Plants



Control

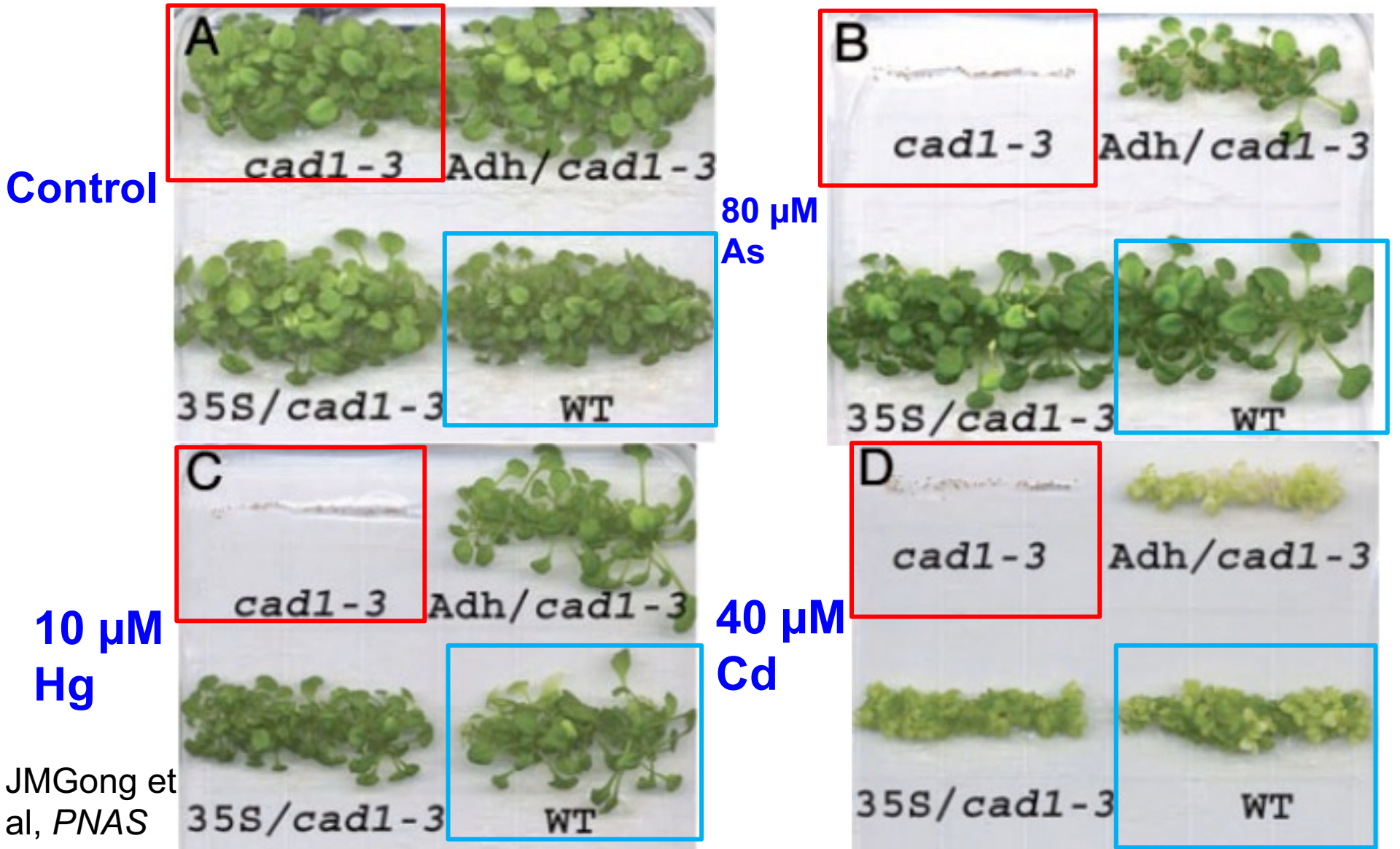
80 μM As

10 μM Hg

40 μM Cd

JMGong et al, *PNAS*

PCS Gene is Essential For Heavy Metal & Arsenic Resistance of Plants



Control

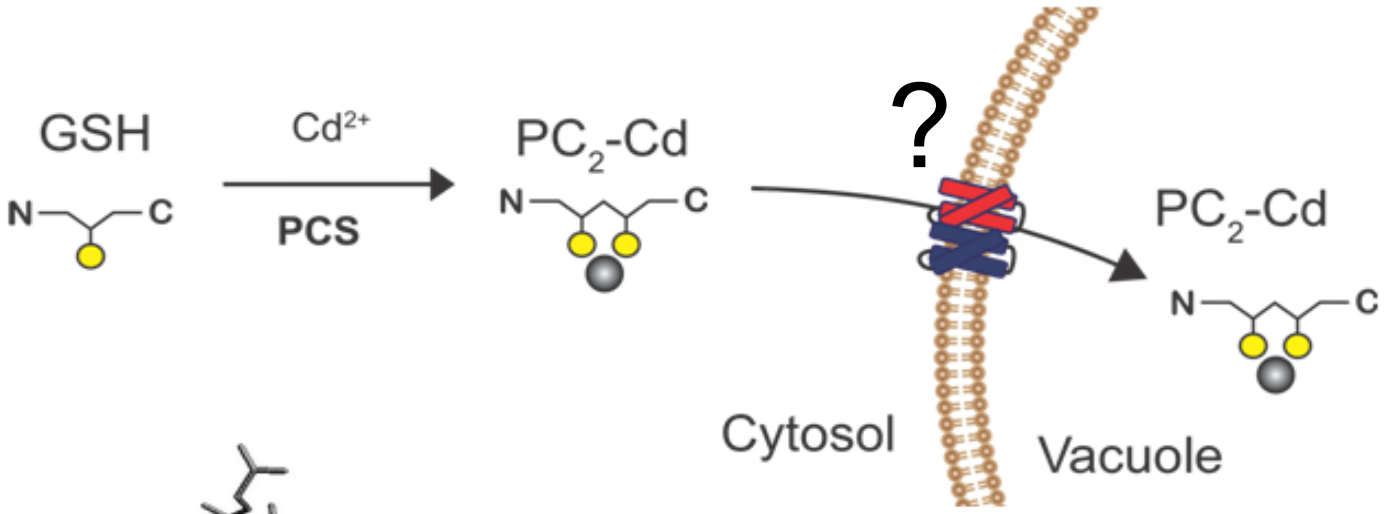
80 μM As

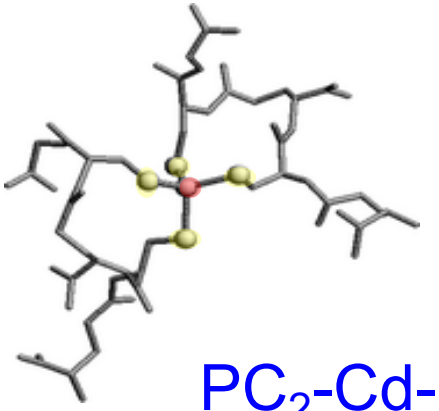
10 μM Hg

40 μM Cd

JMGong et al, *PNAS*

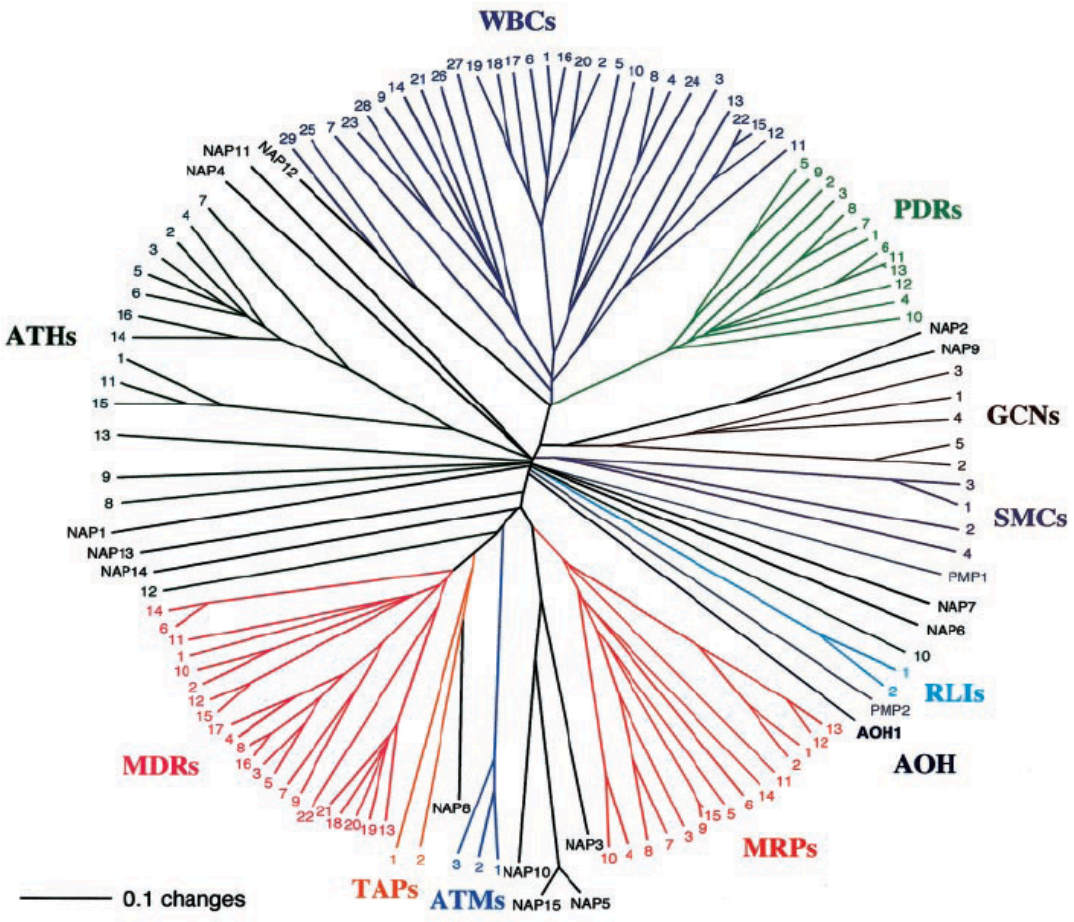
Identity Of The PC Transporter In Plants Was Unknown...

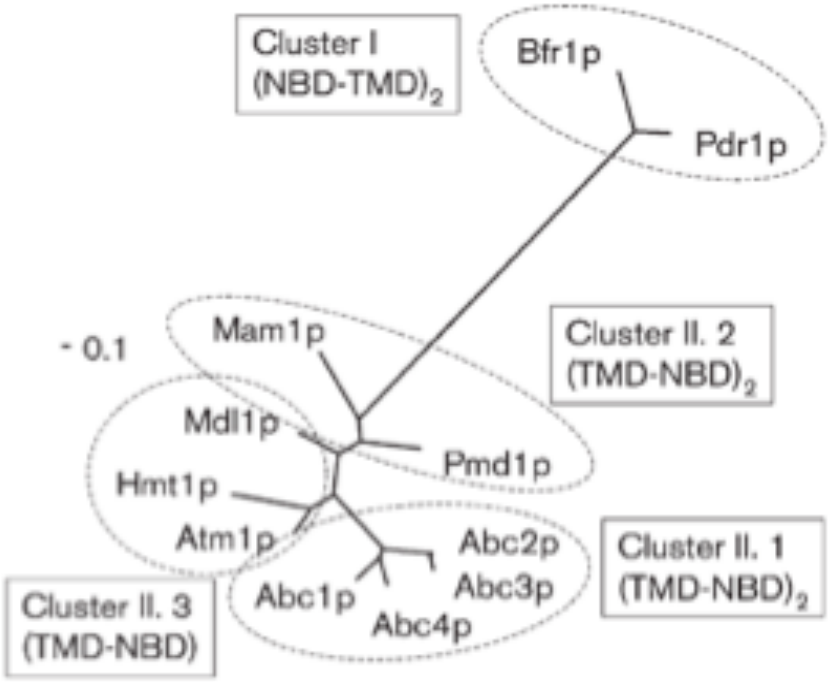




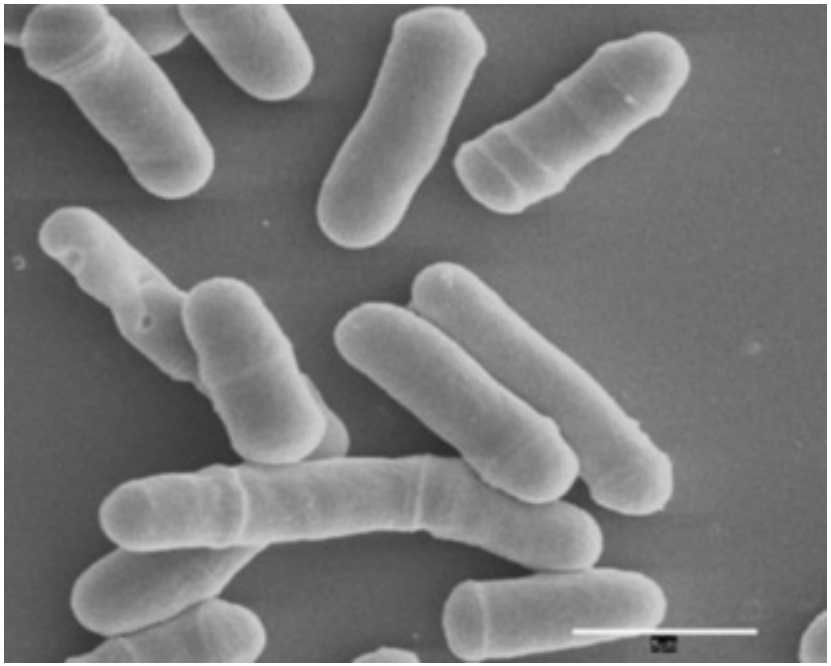
PC_2-Cd-PC_2

ABC Transporters in A. thaliana and S. pombe





Identifying And Characterizing The Elusive Phytochelatin Transporters



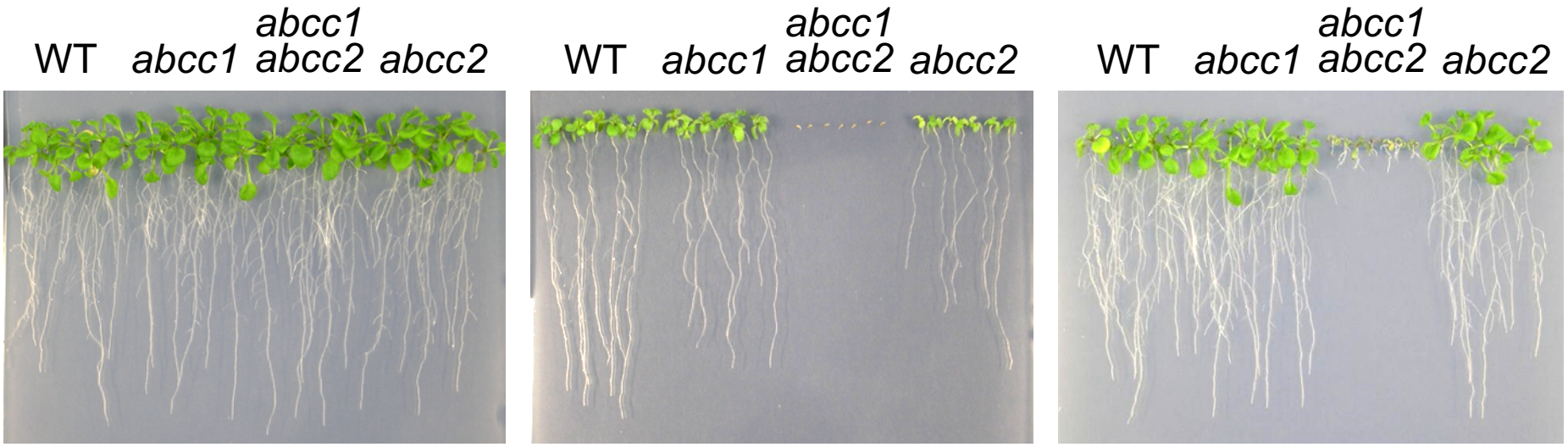
Schizosaccharomyces pombe

Mendoza-Cozatl, Russell, et al.
J. Biol. Chem.

Arabidopsis thaliana

Lee, Park & Mendoza-Cozatl et al., *PNAS*

Arabidopsis abcc1abcc2 is sensitive to arsenic-based herbicides and As(III)



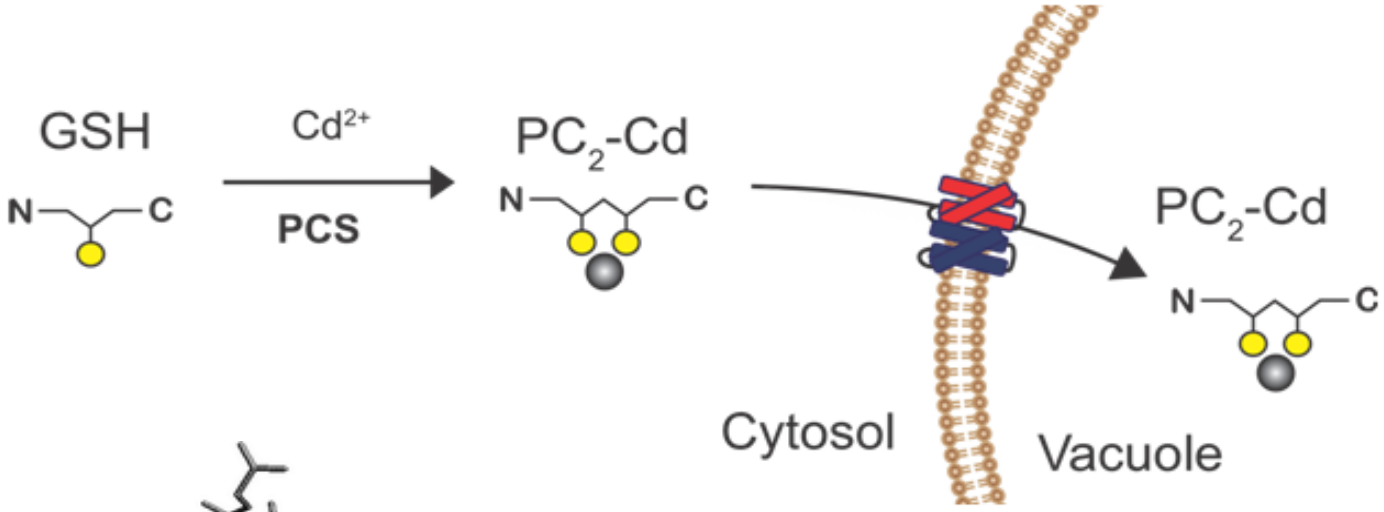
Control

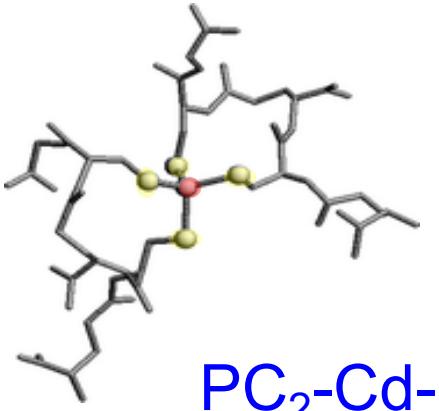
+ DSMA (disodium methyl arsenate
(arsenic-based pesticide)

+ 50 μM (AsIII)

Park / Song / Mendoza-Cózatl *et al*. *PNAS*

Heavy Metal & Arsenic Detoxification Mechanisms

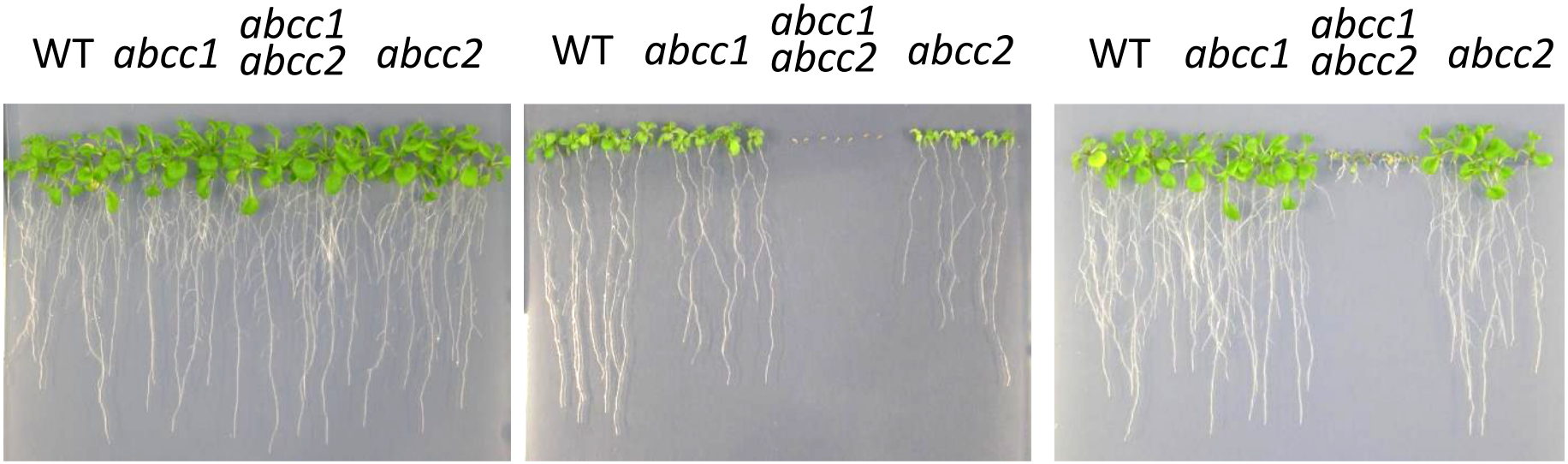




PC_2-Cd-PC_2

Systems Level Approaches to Address Functional Genetic Redundancy

abcc1abcc2 is Sensitive to As(III)



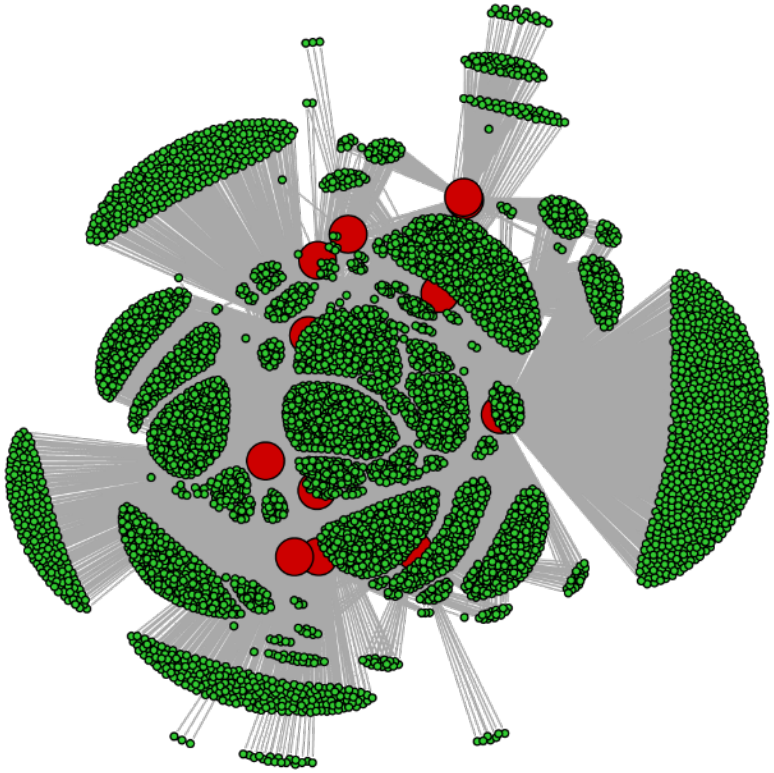
Control

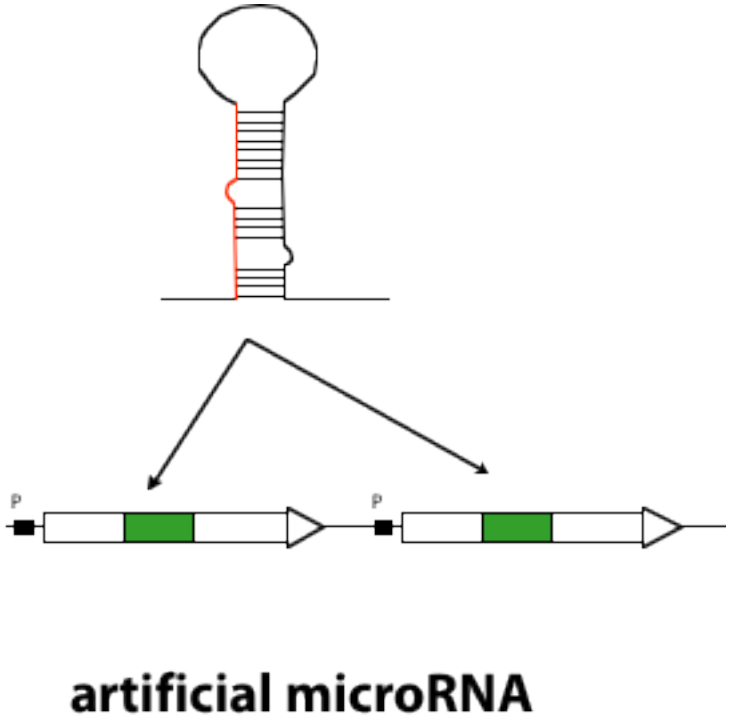
+ DSMA
(arsenic-based pesticide)

+ 50 mM (AsIII)

Song, Park, Mendoza-Cózatl *et al*. *PNAS*

Addressing the Genetic Redundancy Problem

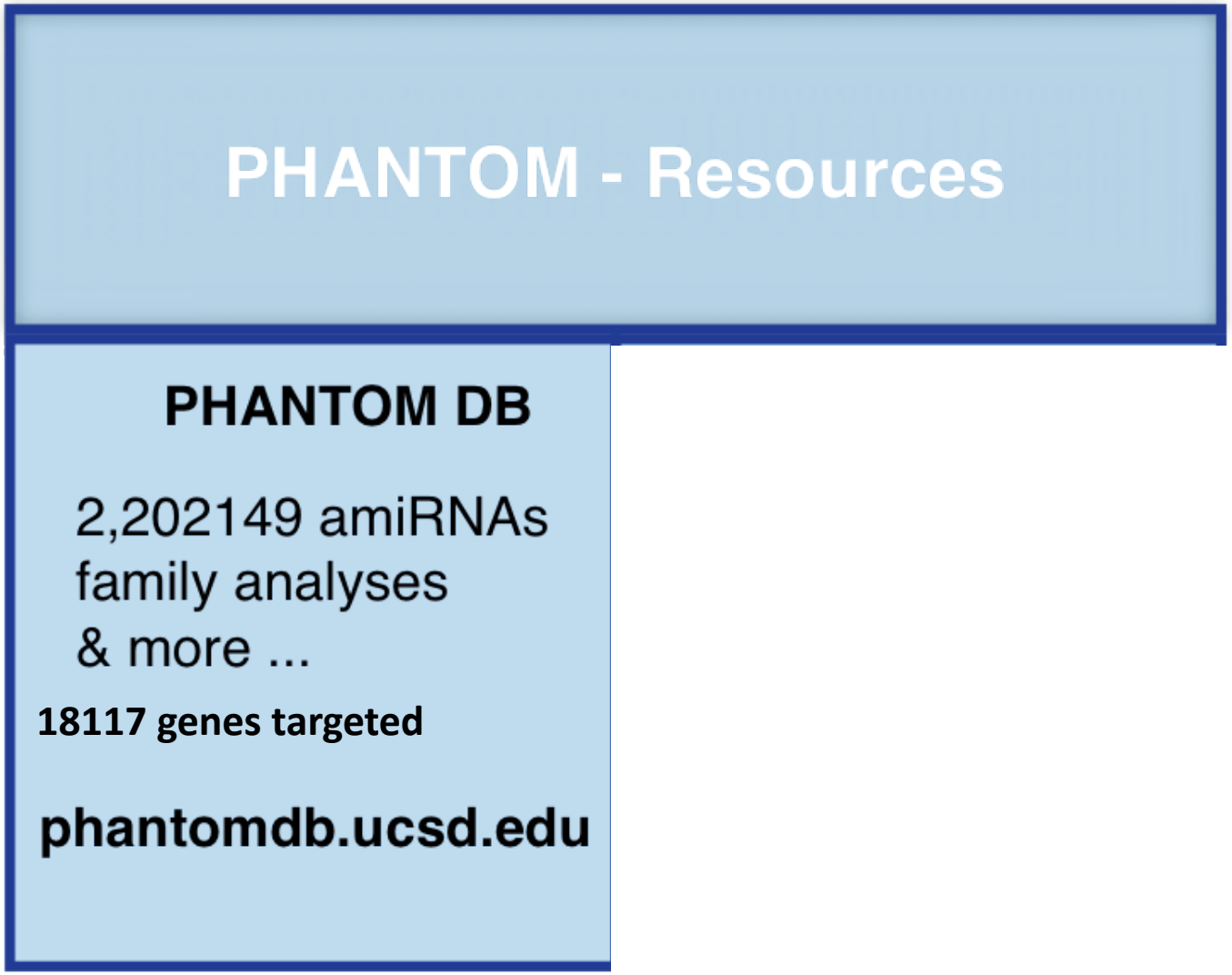




UCSD amiRNA Phantom Resource:
http://phantomdb.ucsd.edu/

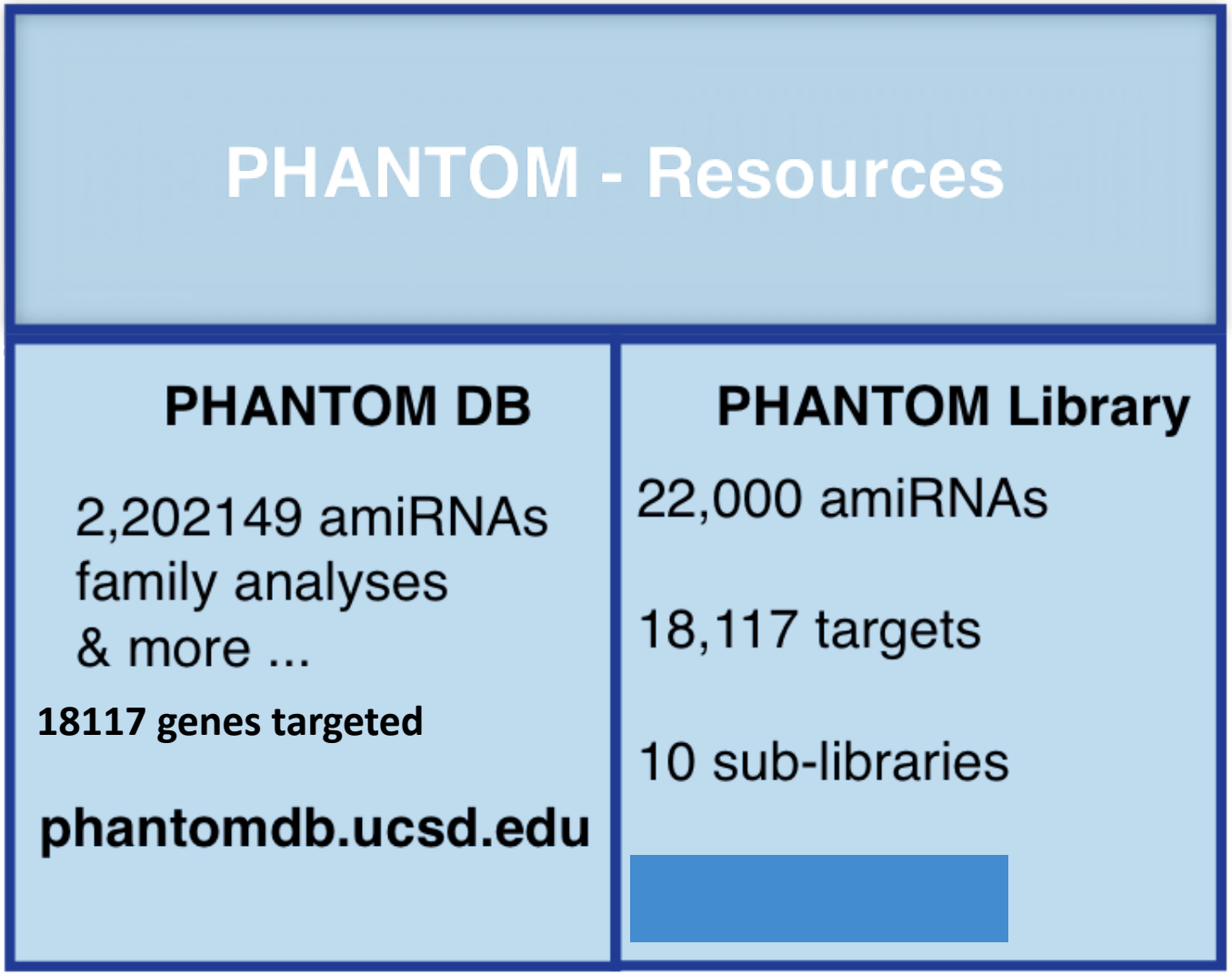
Felix Hauser et al., *Plant Cell*

PHANTOM - Resources: family specific amiRNA library



Felix Hauser et al.

PHANTOM - Resources: family specific amiRNA library

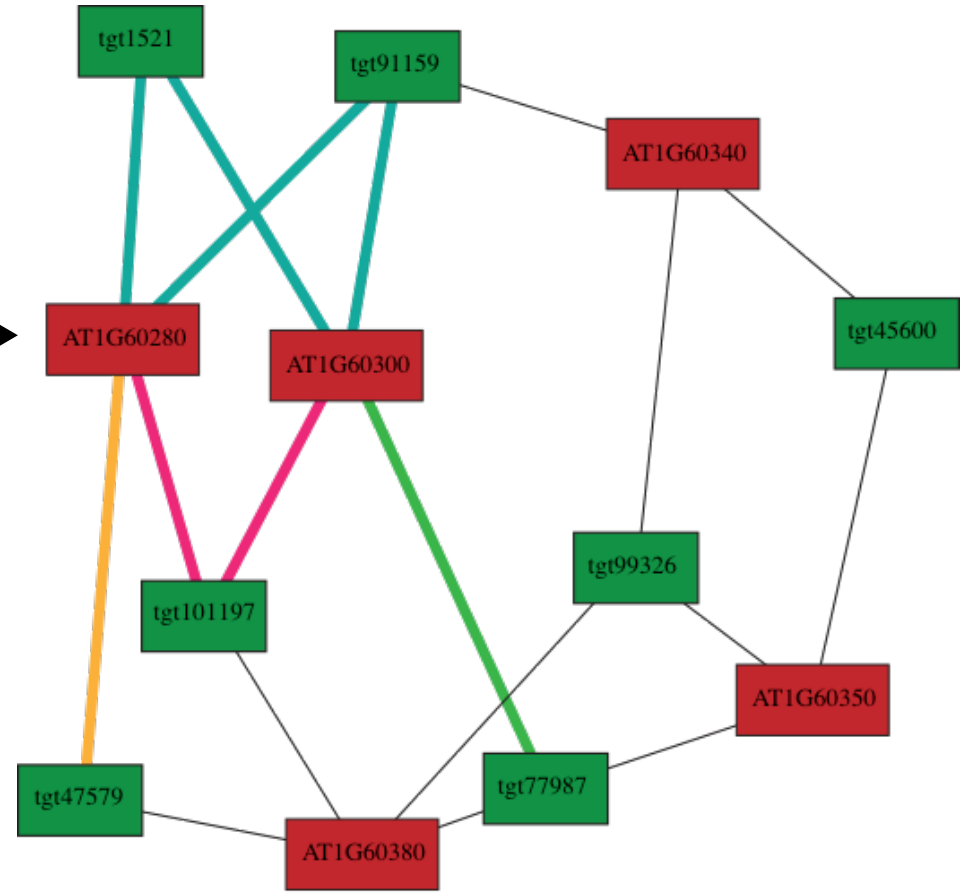


Felix Hauser et al.

Targeting Family Of Homologous Genes

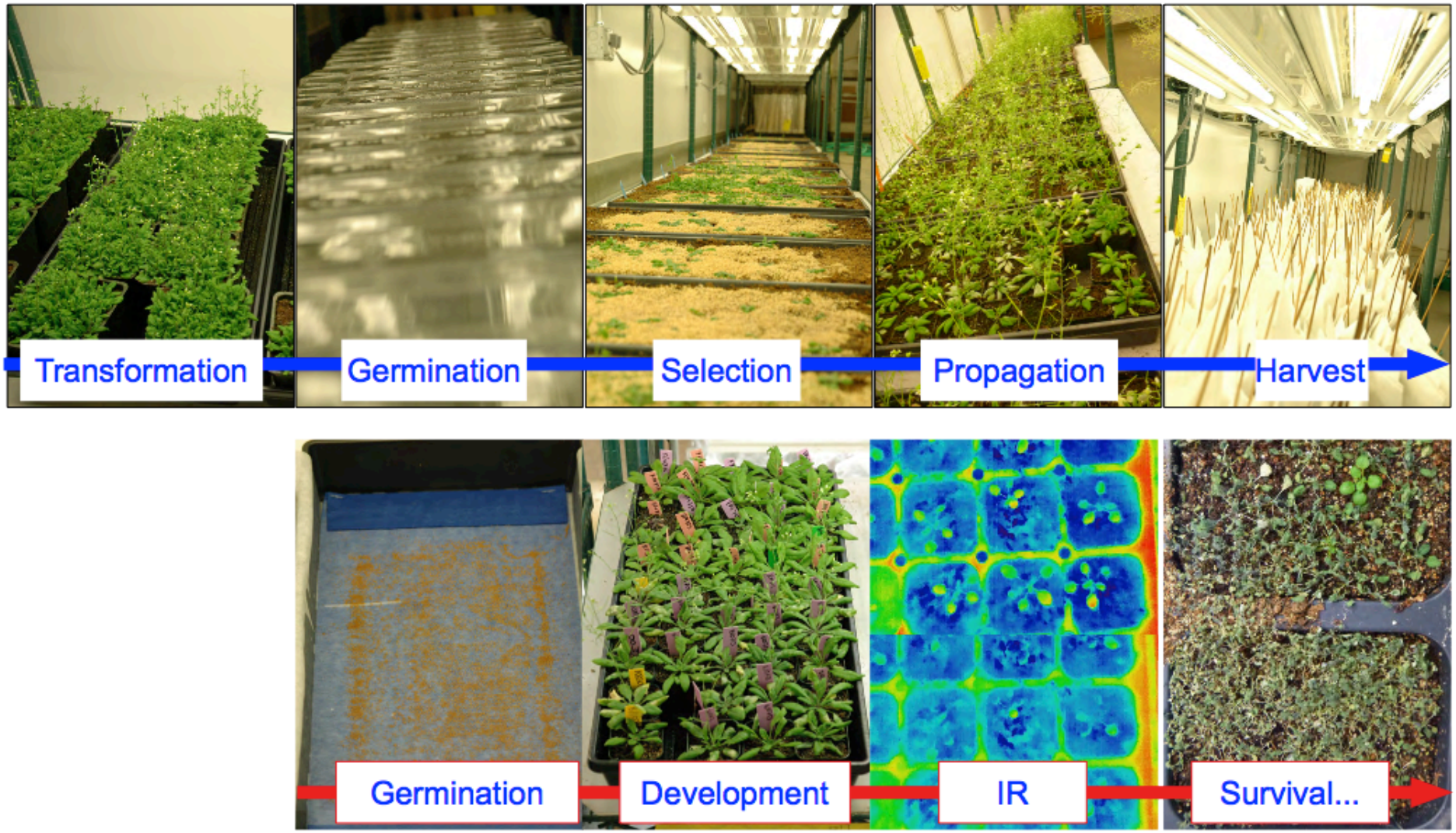
Red: Gene Family Member

Green: amiRNA



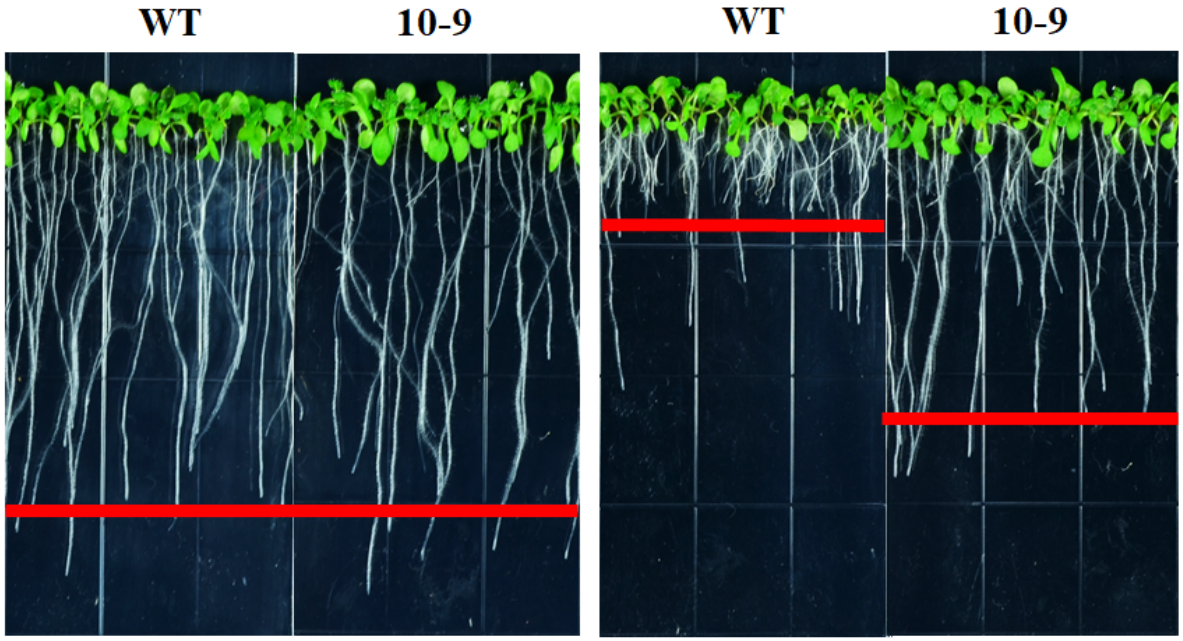
Felix Hauser et al., *Plant Cell*

High-Throughput Screening



Felix Hauser , Paulo.Ceciliato, et al. J.Exp.Bot.2019

Screening amiRNAs On Arsenic



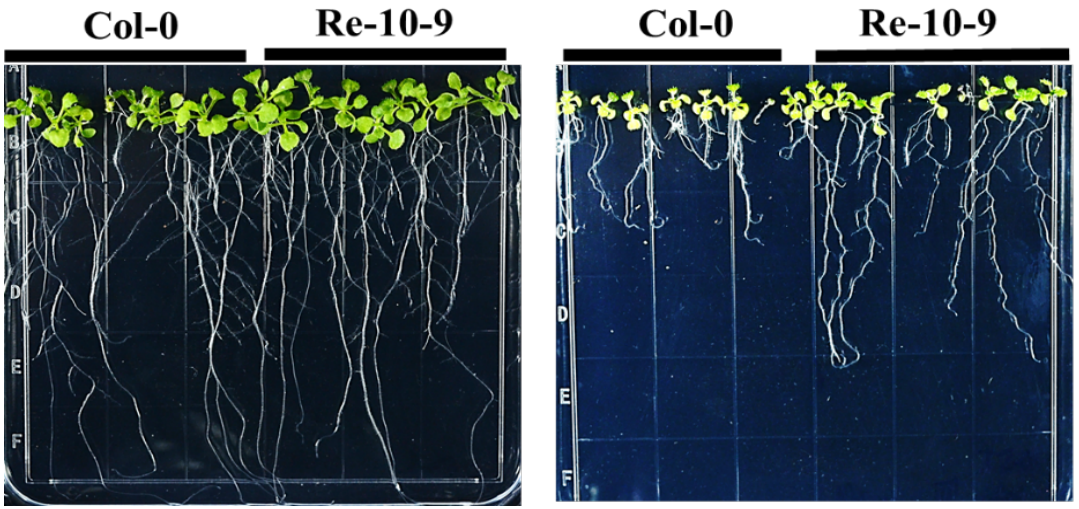
Line 10-9 target genes:

- PHOSPHATE TRANSPORTER A
- PHOSPHATE TRANSPORTER B
- PHOSPHATE TRANSPORTER C

Qingqing Xie et al., unpublished data

10-9 Target Genes

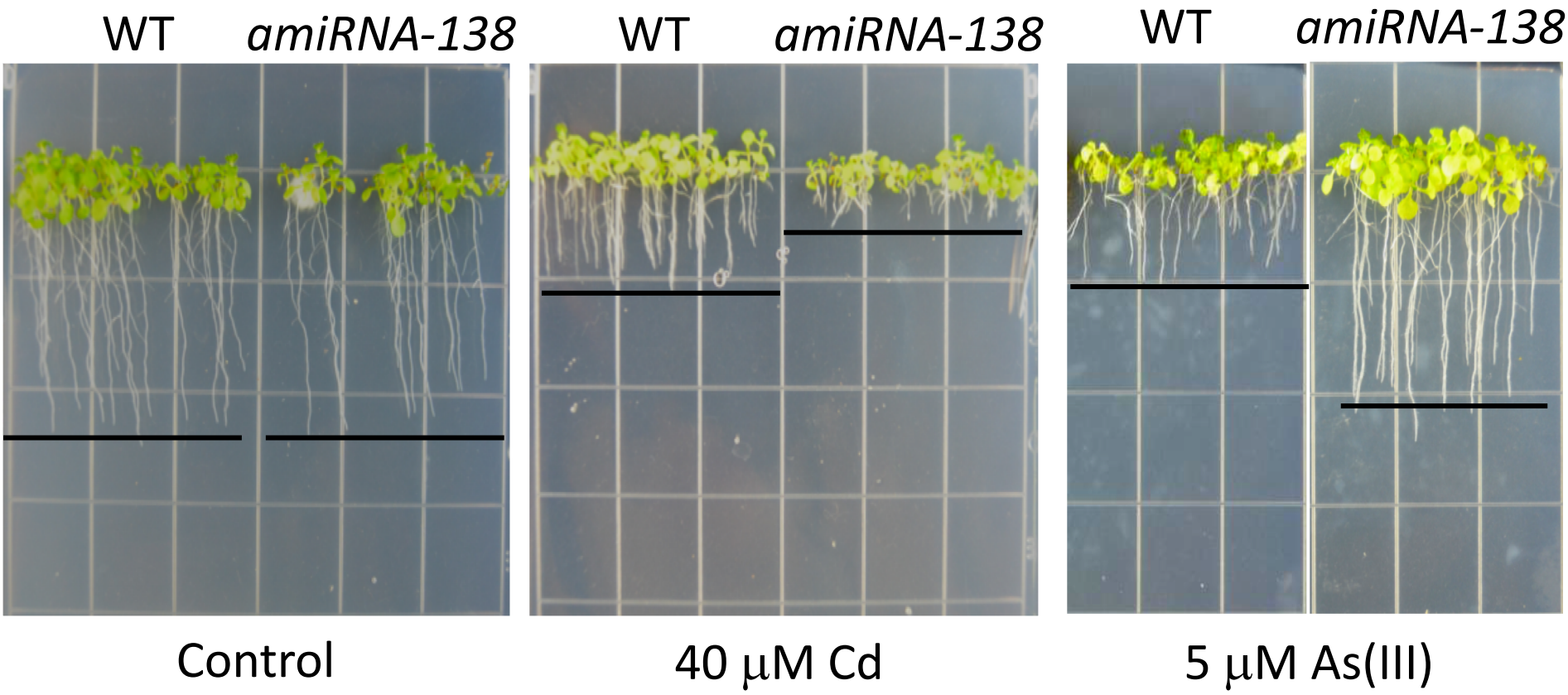
	Gene description



Control

10 µM As (III)

Screening amiRNAs On Cadmium



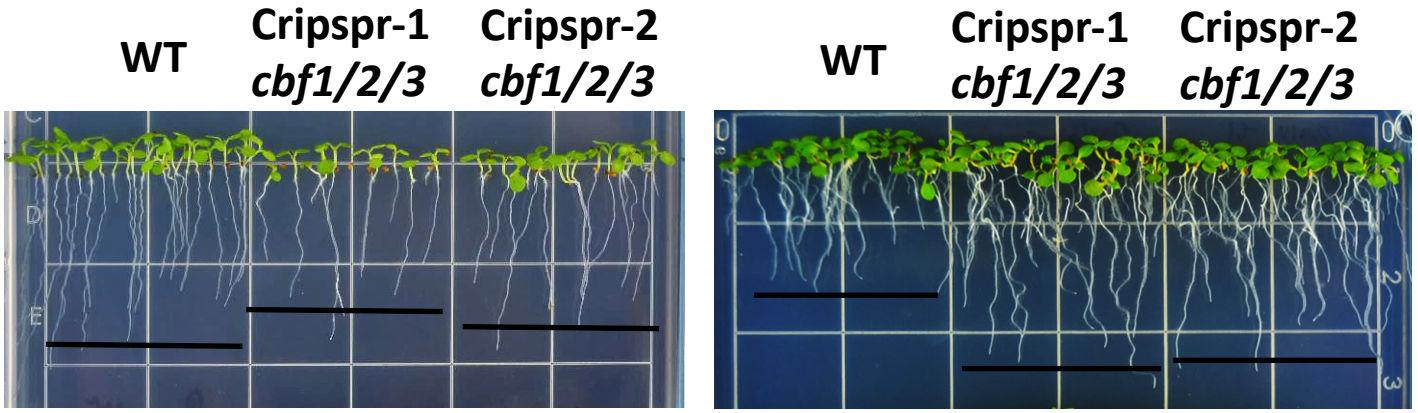
amiRNAs-138 Target Genes

amiRNA Sequence:

TAACTTCTCATCCGCACACCG

Targeted genes	Gene description	Hybridization energy	Match percentage
AT2G44940	ERF034	-49.82 kcal/mol	100.00%
AT4G25480	CBF3	-43.49 kcal/mol	87.29%
AT3G60490	ERF035	-42.40 kcal/mol	85.11%
AT4G25470	CBF2	-38.38 kcal/mol	77.04%
AT4G25490	CBF1	-38.38 kcal/mol	77.04%
AT5G51990	CBF4	-36.05 kcal/mol	72.36%

CBF1,CBF2,CBF3 CRISPR triple deletion Shows Increased As resistance



Control

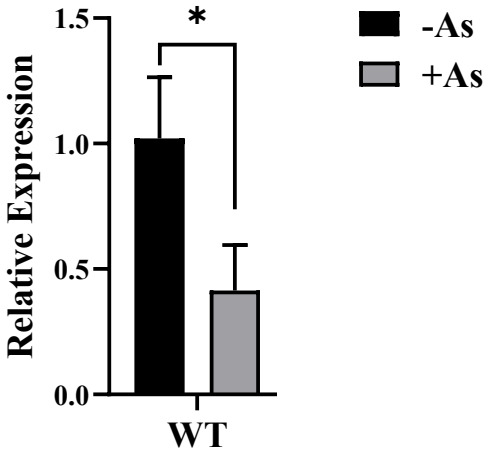
10μM As(III)

Q. Yu et al., unpublished

CBF1,CBF2,CBF3 and CBF4 Transcription Factor DAP-seq Target Genes

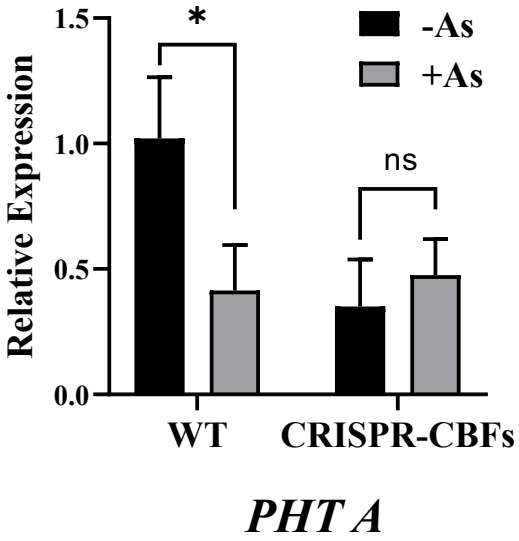
CBFs	Target genes	Gene description
CBF1	PHT C	phosphate transporter C
	PHT L	phosphate transporter L
	PHT E	phosphate transporter E
	PHT F	phosphate transporter F
	PHT G	phosphate transporter G
	PHT H	phosphate transporter H
	PHT I	phosphate transporter I
	PHT J	phosphate transporter J
CBF2	PHT C	phosphate transporter C
	PHT F	phosphate transporter F
	PHT G	phosphate transporter G
	PHT I	phosphate transporter I
	PHT J	phosphate transporter J
CBF3	PHT F	phosphate transporter F
	PHT G	phosphate transporter G
	PHT I	phosphate transporter I
CBF4	PHT A	phosphate transporter A
	PHT C	phosphate transporter C
	PHT D	phosphate transporter D
	PHT E	phosphate transporter E
	PHT F	phosphate transporter F
	PHT G	phosphate transporter G
	PHT I	phosphate transporter I
	PHT J	phosphate transporter J
	PHT K	phosphate transporter K

Expression of Phosphate transporter A

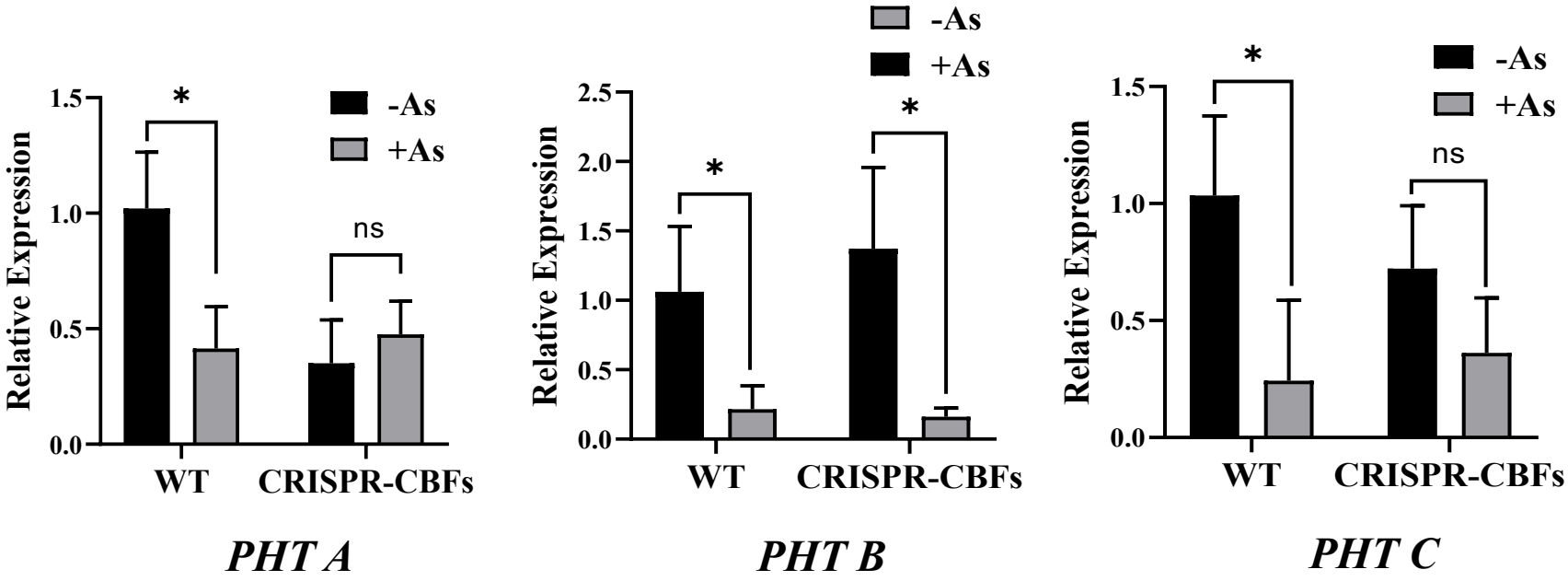


PHT A

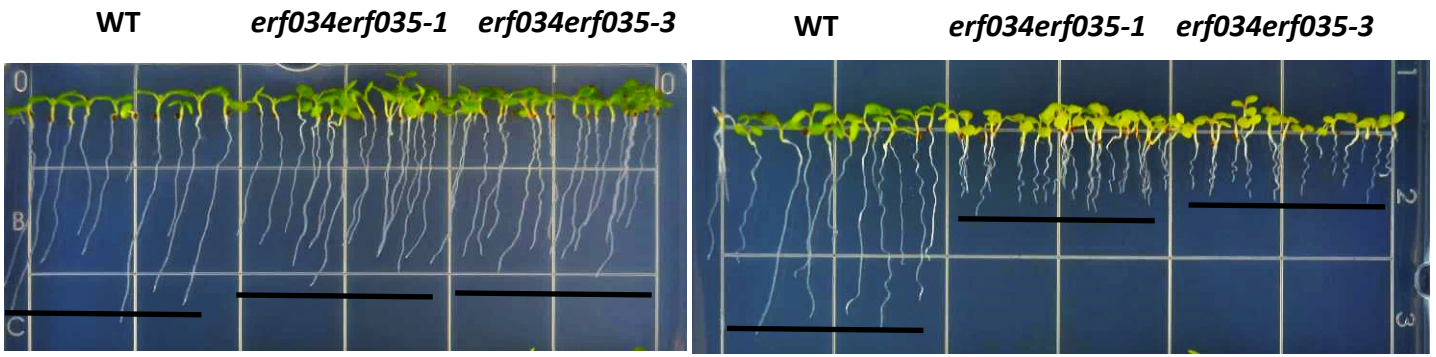
Expression of Phosphate transporter A in *CBF1/2/3* triple mutant



The expression of Phosphate transporters in *CBF1/2/3* triple mutant



Erf034 erf035 double mutant Shows Increased Cd Sensitivity



Minimal medium

Minimal medium+30μM Cd

Qi Yu, Qingqing Xie et al., unpublished data

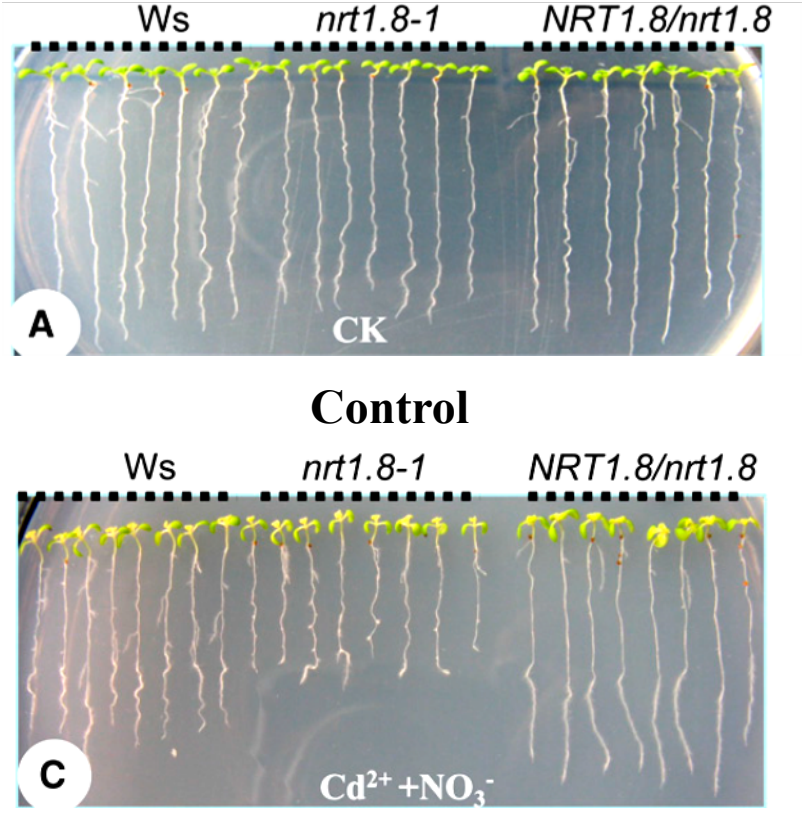
CHIP-seq-like (DAP-seq) analysis of candidates *erf034 and erf035 t*arget genes

Gene Code	Gene Symbol	Gene description	Fold change	p-value
AT4G21680	NRT1.8	NITRATE TRANSPORTER 1.8	29	0.0406
AT4G14680	APS3	Pseudouridine synthase/archaeosine transglycosylase-like family protein	12.3	0.0401
AT1G62300	WRKY6	WRKY family transcription factor	5.6	0.0327
AT4G01950	GPAT3	glycerol-3-phosphate acyltransferase 3	4.3	0.0367
AT4G17500	ERF-1	ethylene responsive element binding factor 1	3.9	0.0483
AT3G12580	HSP70	heat shock protein 70	3.4	0.0327
AT1G08920	ESL1	ERD (early response to dehydration) six-like 1	3.3	0.0483
AT2G32560	AT2G32560	F-box family protein	3	0.0483
AT1G78820	AT1G78820	D-mannose binding lectin protein with Apple-like carbohydrate-binding domain-containing protein	2.9	0.04
AT3G25230	ROF1	Rotamase FKBP 1	2.7	0.0401
AT4G26080	ABI1	Protein phosphatase 2C family protein	2.6	0.0483
AT3G47960	GTR1	Major facilitator superfamily protein	2.5	0.0483
AT2G18690	AT2G18690	transmembrane protein	2.4	0.05
AT2G41800	AT2G41800	Protein of unknown function, DUF642	2.4	0.0483
AT2G21130	AT2G21130	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	2.3	0.0418
AT2G41410	AT2G41410	Calcium-binding EF-hand family protein	2.3	0.0483
AT5G16600	MYB43	myb domain protein 43	2.3	0.0483
AT3G46130	MYB48	myb domain protein 48	2.2	0.0485

Table. Genes significantly ($p<0.05$) induced >2fold after 2 hours of exposure to 50 μM Cd^{2+} in A. thaliana roots (the target genes from DAP-Seq)

Qi Yu et al., unpublished data

NRT1.8 mutant Shows Increased Cd Sensitivity



J. Li et al. Schroeder JI, Gong JM *et al*. *The Plant Cell*

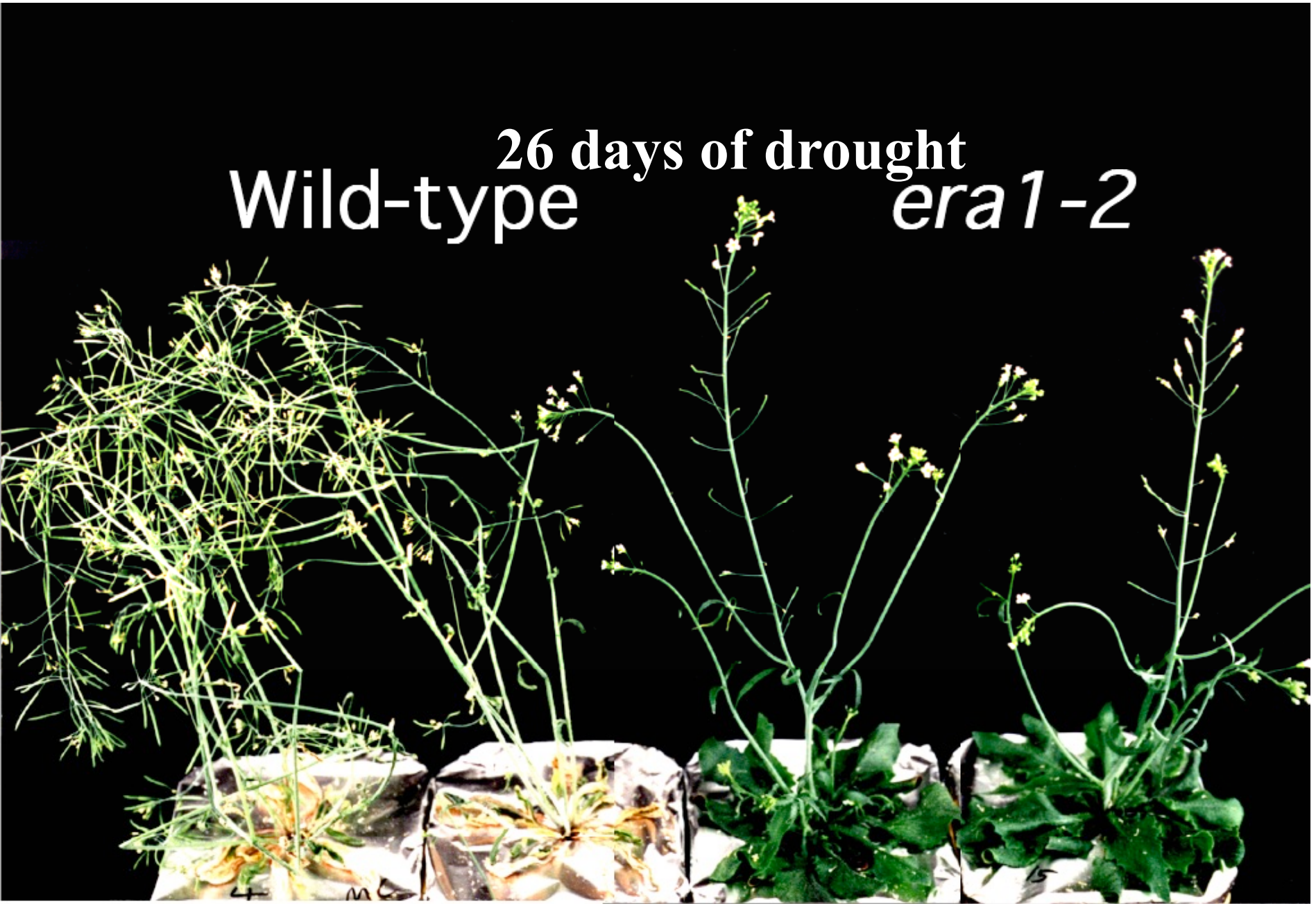
Summary

Large gene families in plants disproportionally limit gene discovery and forward genetic screens

Development of an omics Resource to address the numerous large gene families in plants with overlapping gene functions

New powerful screen of functionally redundant gene space is leading to identification of new genes/gene families and network principles that function in heavy metal and arsenic resistance

Translating from Lab to Field



Z.M. Pei et al. *Science*

Iron King Mine/Humboldt Smelter Superfund Site

Buffalo Grass / Quailbush (Atriplex Lentiformis)

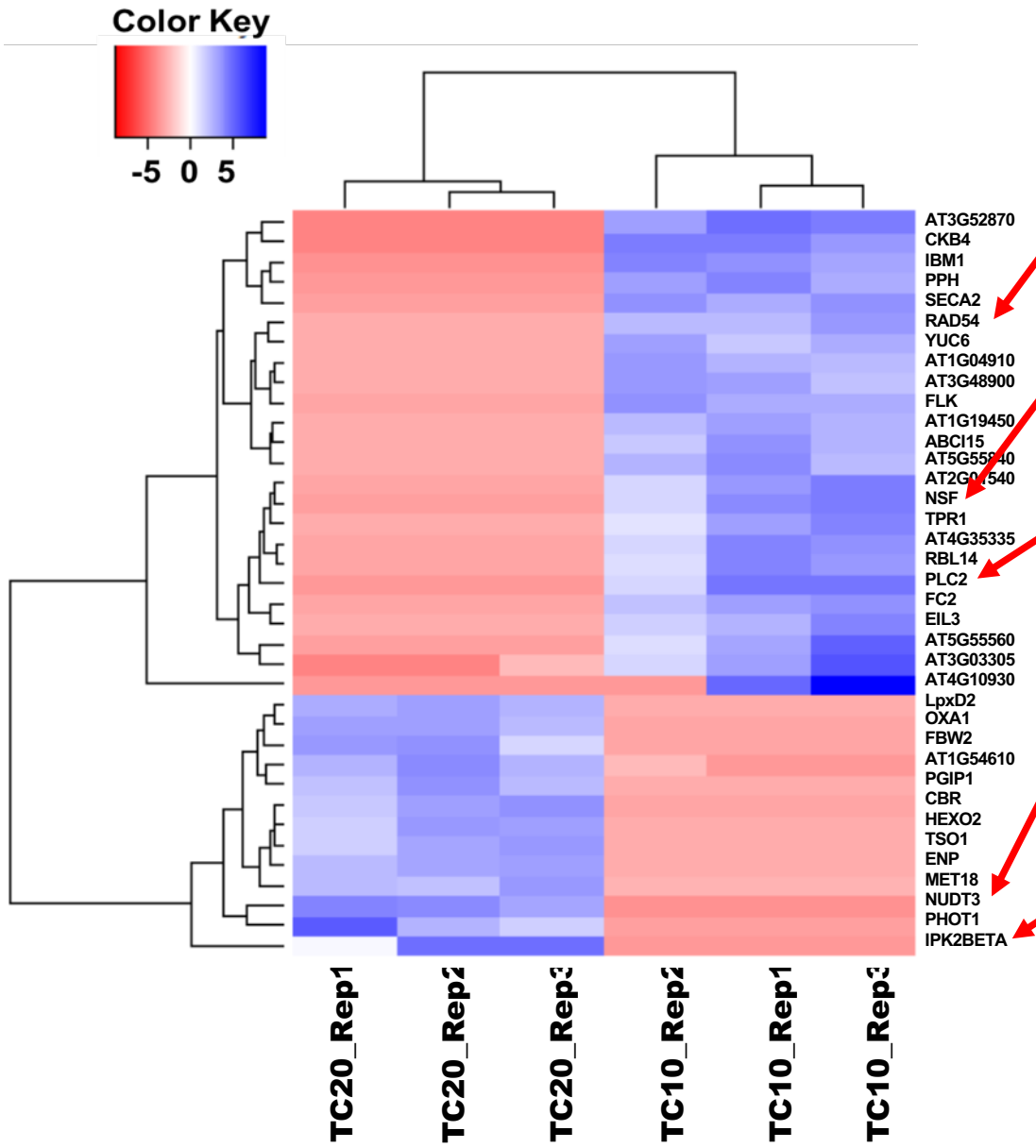
Source: Dr. Raina Maier U. Arizona

The sample list of RNA-seq of Atriplex lentiformis

Sample ID	Treatment	Sample type
TC10-2L	Tailings + 10 % Compost	Leaves
TC10-2S	Tailings + 10 % Compost	Shoot
TC10-2R	Tailings + 10 % Compost	Root
TC15-2L	Tailings + 15 % Compost	Leaves
TC15-2S	Tailings + 15 % Compost	Shoot
TC15-2R	Tailings + 15 % Compost	Root
TC20-3L	Tailings + 20 % Compost	Leaves
TC20-3S	Tailings + 20 % Compost	Shoot
TC20-3R	Tailings + 20 % Compost	Root
PS-2L	Potting Soil	Leaves
PS-2S	Potting Soil	Shoot
PS-2R	Potting Soil	Root

Four biological replications for each treatment (Total 48 samples) Qi Li (UCSD), Priyanka Kushwaha (U. Arizona SRC), RNA-seq with Ron Evans's lab (Salk Institute), Analysis with Alexandria Tran & Chris Benner (UCSD)

Differentially expressed quailbush genes in the root samples comparing two treatments: 10 vs. 20% compost amendment



RAD54: DNA repair/recombination protein

NSF: AAA-type ATPase family proteins that play a role in adaptation to salt stress

PLC2: phospholipase C2 is major membrane phospholipid that plays a role in response to abiotic stress

NUDT3: Plays a role in protection against oxidative DNA and RNA damage in plant cells

IPK2BETA: Inositol polyphosphate kinase 2 beta plays a role in plant tolerance to abiotic stress

Priyanka Kushwaha(U. Arizona SRC) & Qi Li (UCSD SRC), unpublished

Consumer Reports:
High Arsenic Levels in Juice

January 2012

10% of apple and grape juice samples tested from the U.S. had arsenic levels exceeding federal standards for drinking water

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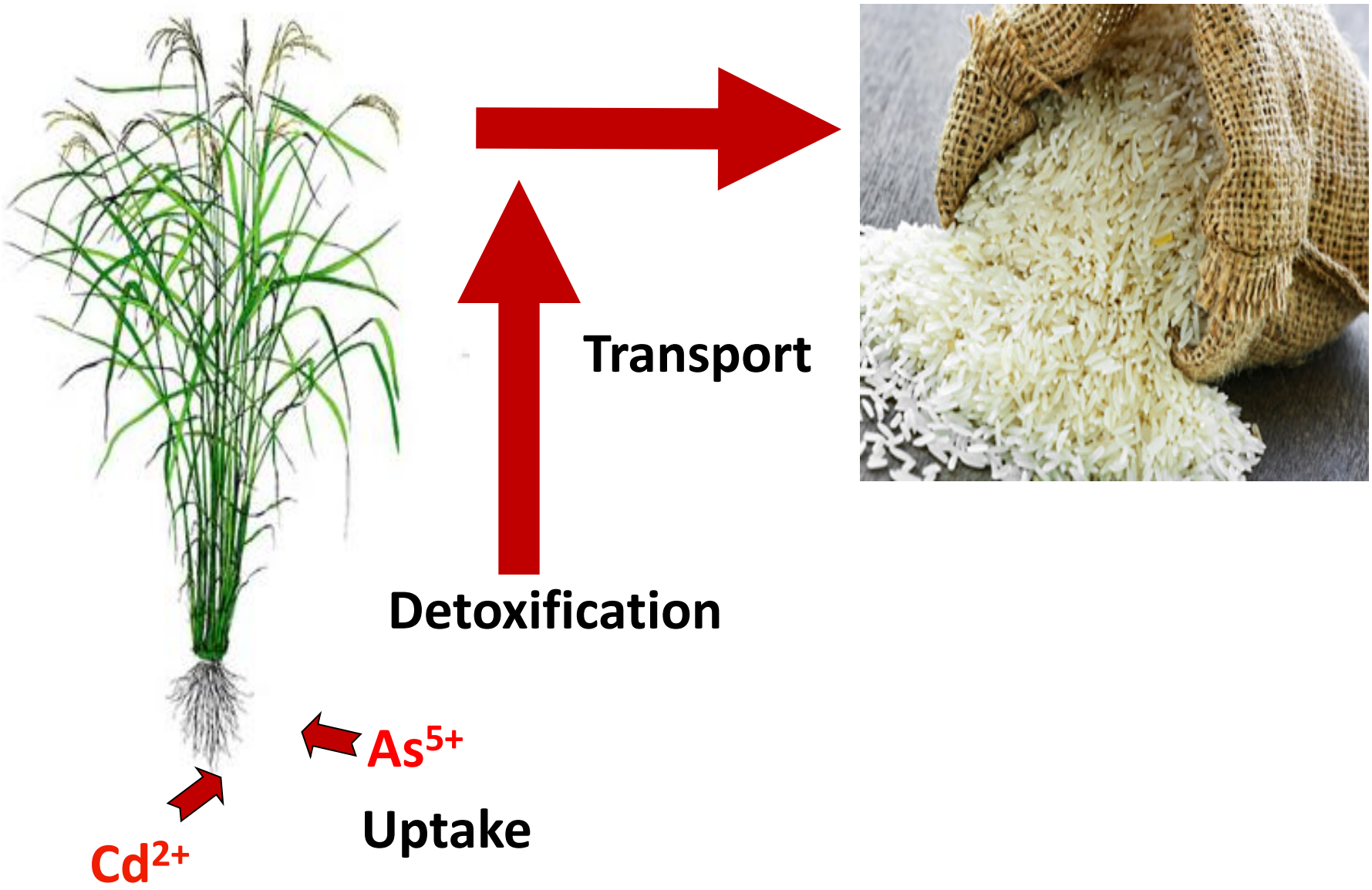
Consumer reports, Jan 2012. Arsenic in Juice. Retrieved Dec. 2012

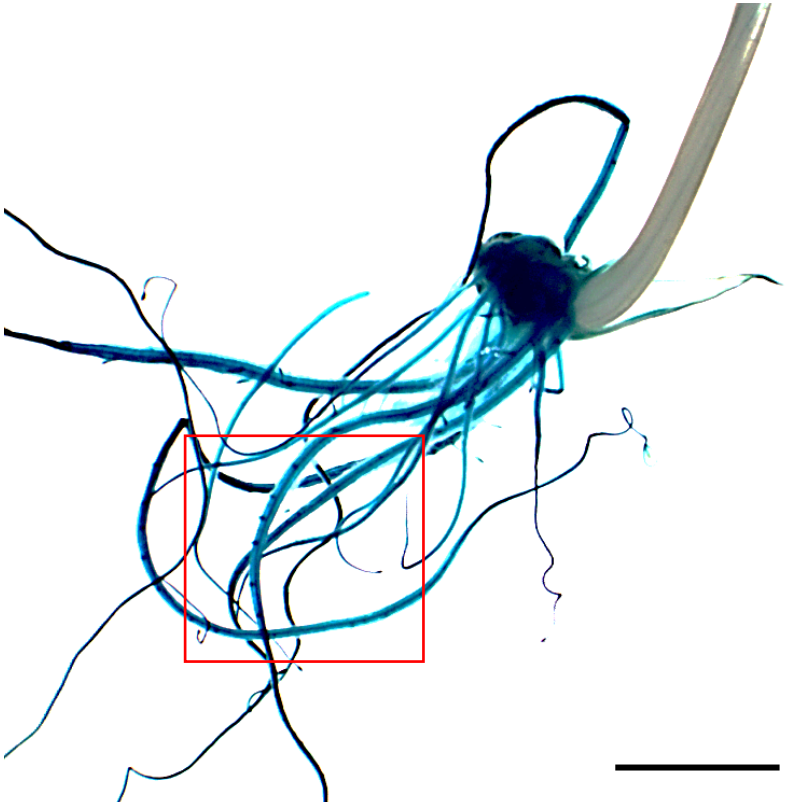
Food Chain Contamination From Plant Uptake



Promoters for Root Expression

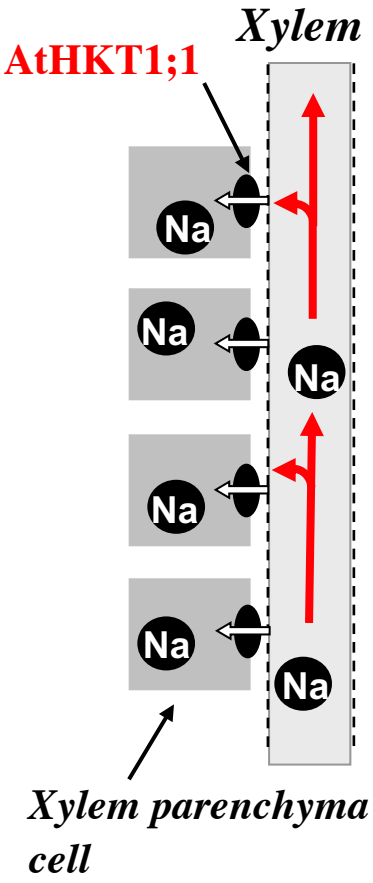
Arabidopsis thaliana

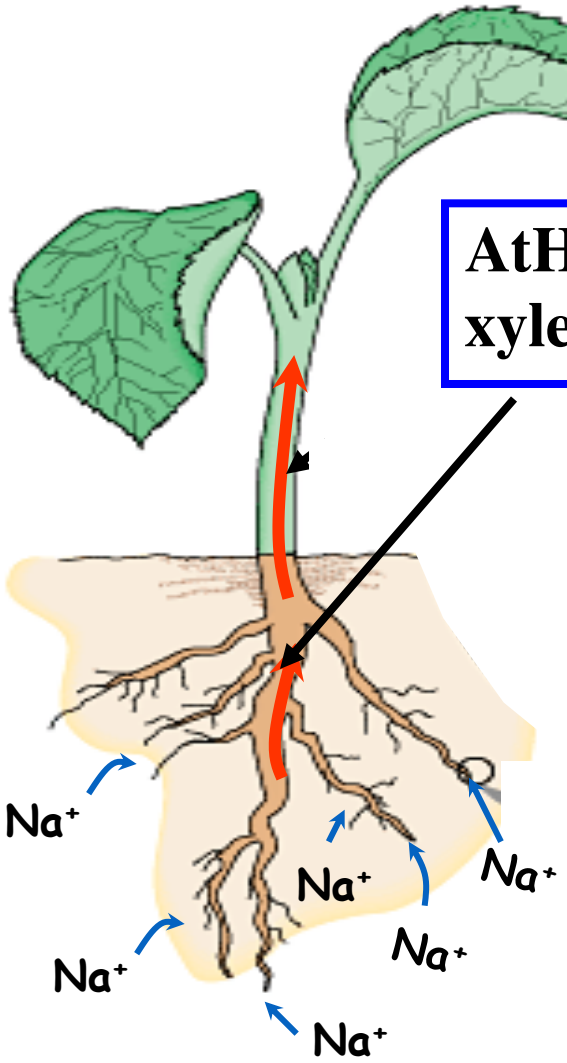
Oryza sativa

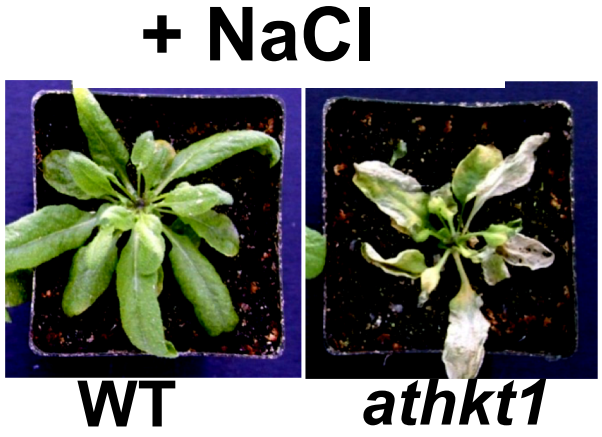


K. Jung, Unpublished Data

HKT Transporters Protect *Arabidopsis* and Rice Leaves from Salinity Stress

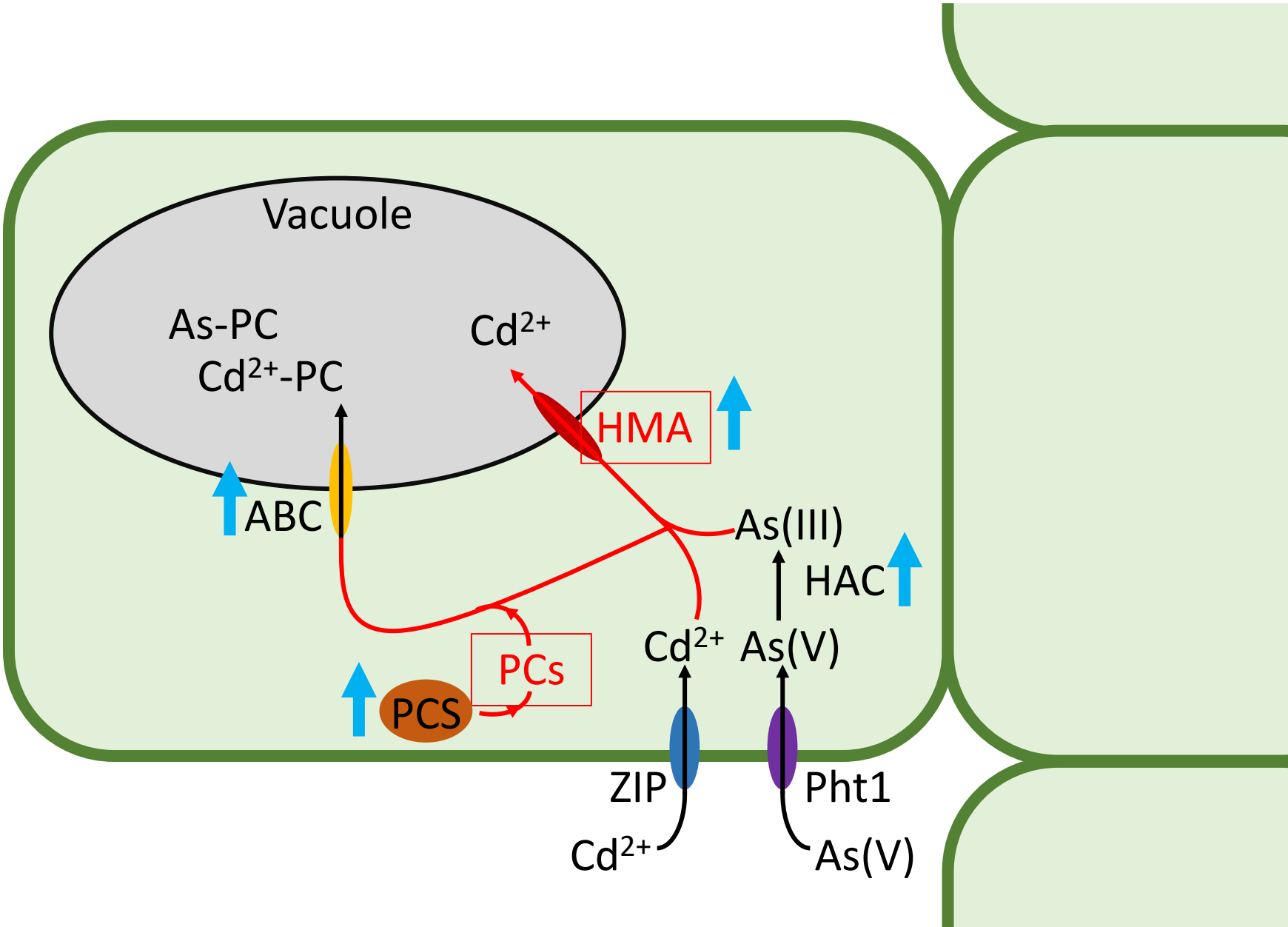






Rubio et al. 1995 *Science*; Uozumi et al., *Pl. Phys*. Mäser *et al*. *PNAS*; Ren Nat. Gen. 2005;Sunarpi *Pl. J* 2005; R. Munns et al & M. Gilliham *Nat Biotech* 2012

Heavy Metal Uptake In Plants



Rice Transgenics Status

- Independent rice lines expressing expressing heavy metal and arsenic sequestration mechanisms in roots.
- Additional transformations are in progress.

Nipponbare WT

Transgene 1

Transgene 2

Qi Yu, Andrew, Cooper Unpublished Data; transformation An & P. Ronald

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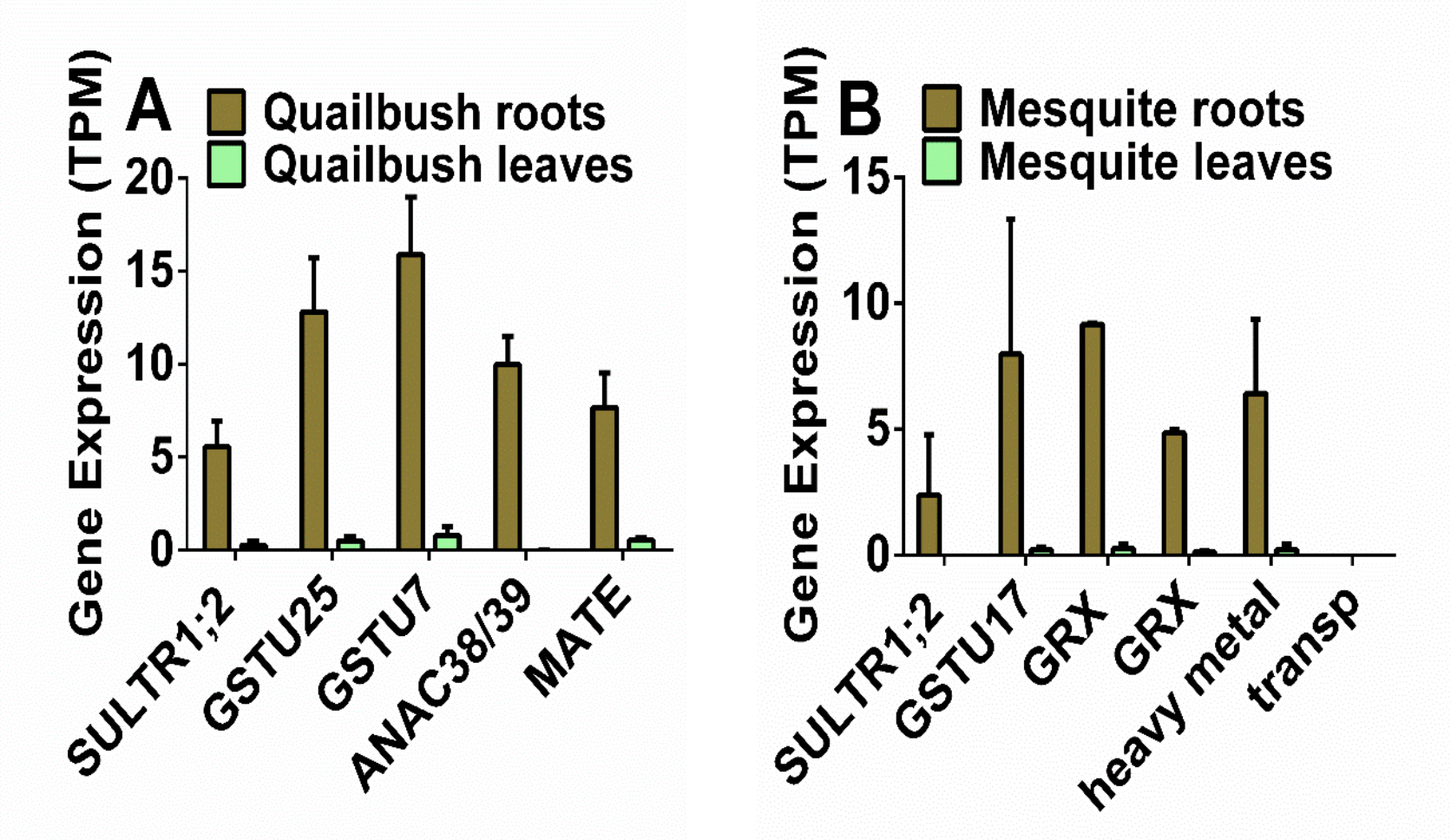
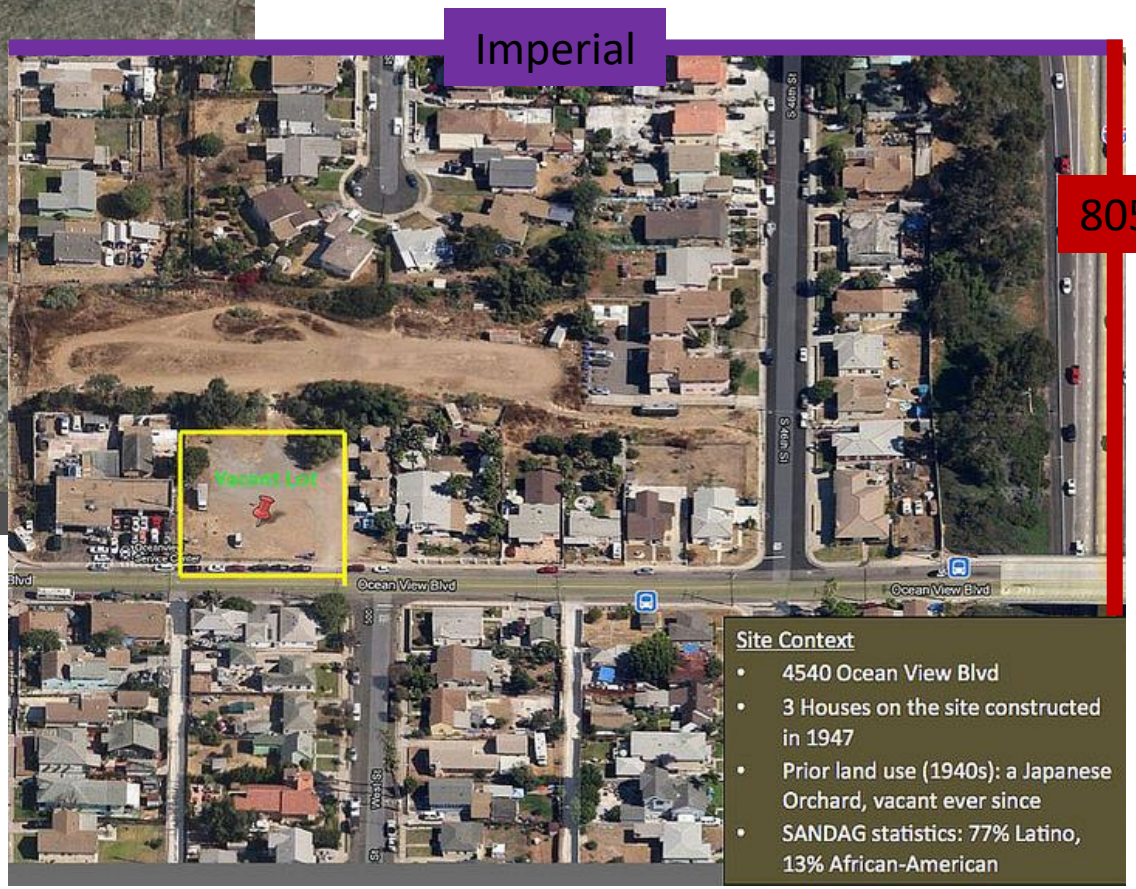
NIEHS – SRP

RNA-seq of Quailbush (Atriplex Lentiformis) and Mesquite Transcriptome



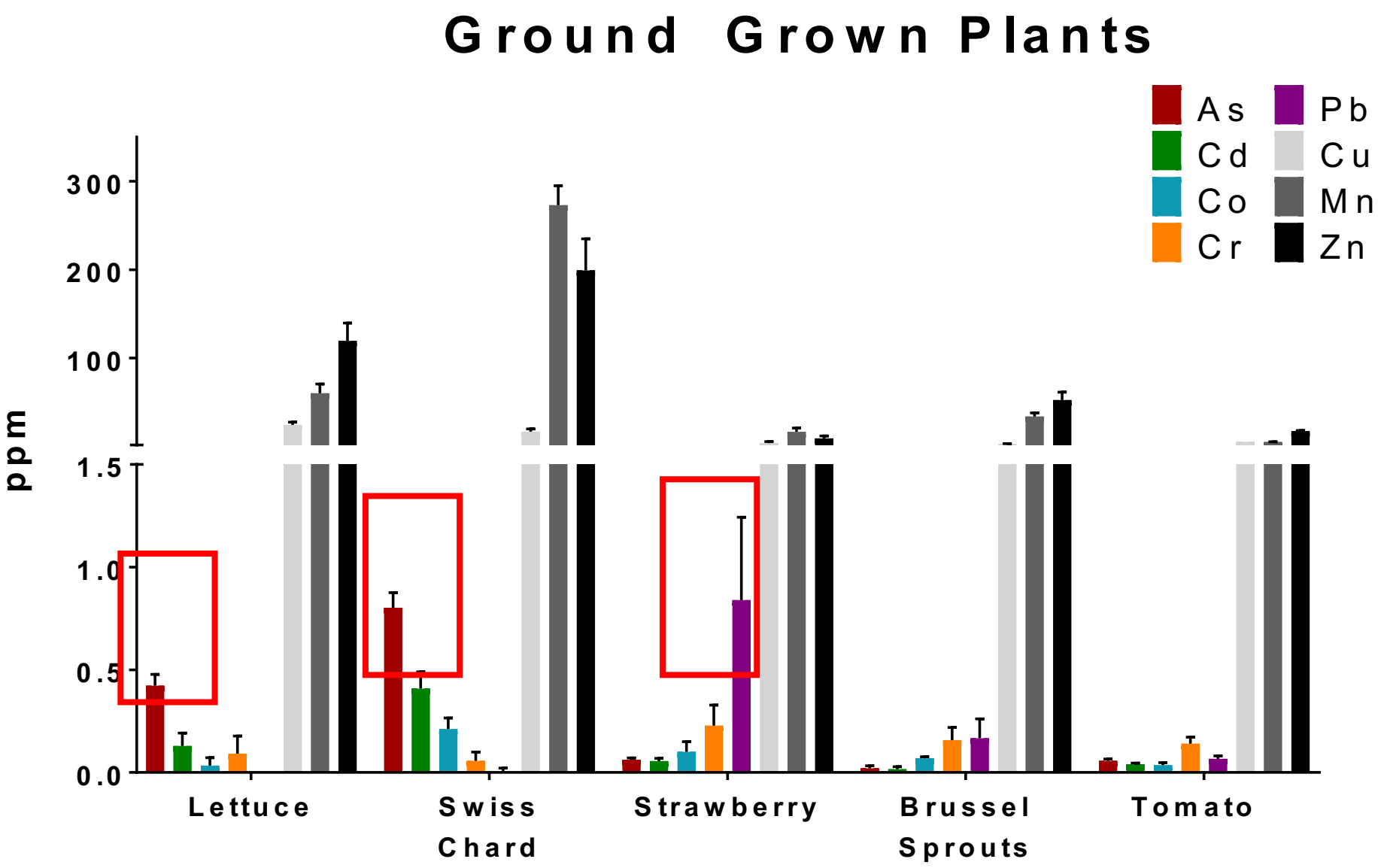
C. Engineer, R. Maier, R. Evans, C. Benner, unpublished

Ocean View Growing Grounds (OVGG)



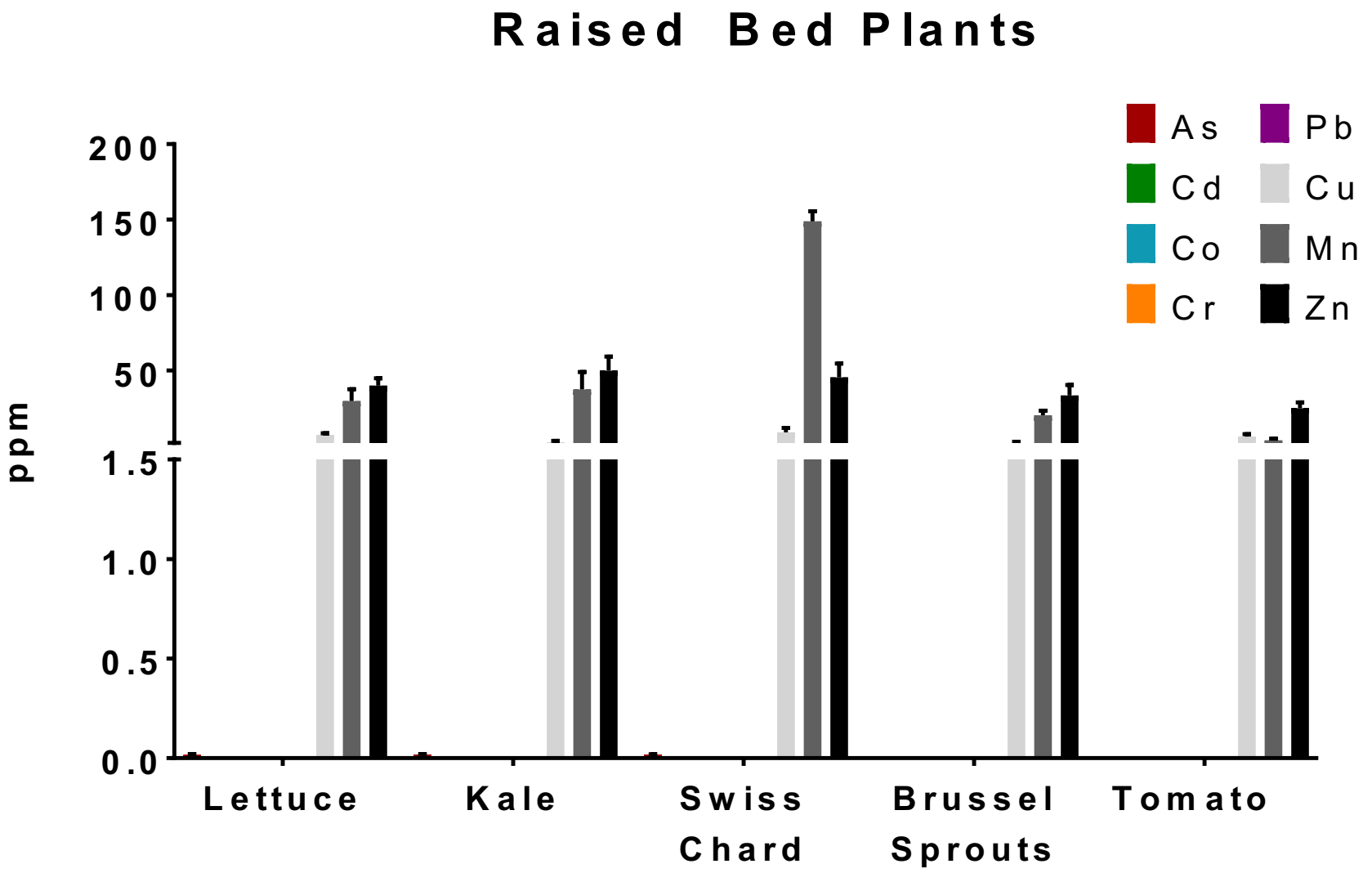
Collaboration with
Keith Pezzoli
CEC core

OVGG-Ground Grown Samples



OVGG-Raised Beds

OVGG-Raised Bed Samples



Iron King Mine/Humboldt Smelter Superfund Site

Atriplex lentiformis

Source: Dr. Raina Maier U. Arizona

Agricultural Contamination

Integration of Primary Screening Data into a Phenotype to Genotype Network