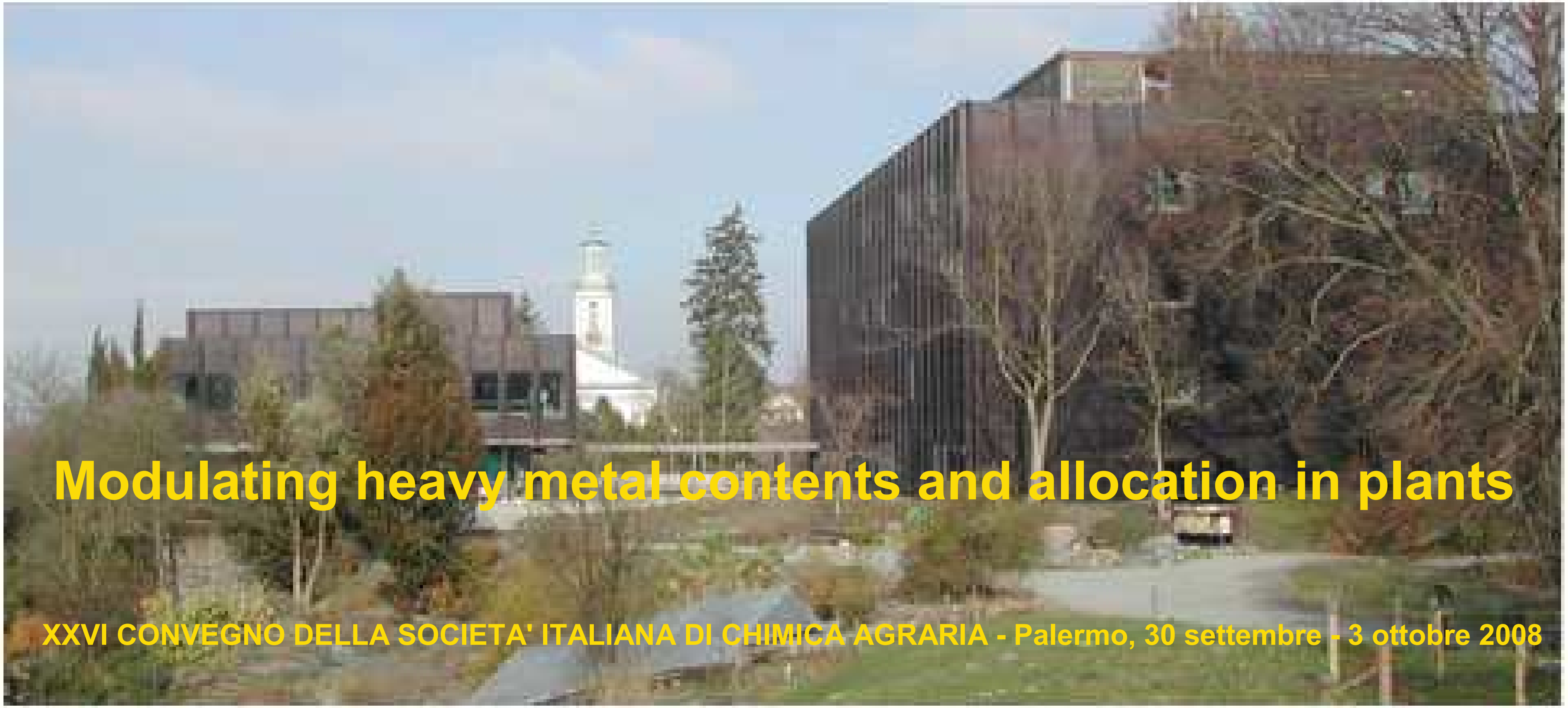
Institute of Plant Biology
Laboratory for Molecular Plant Physiology
POSTECH-UZH Cooperative Laboratory
University Zurich



# Modulating heavy metal contents and allocation in plants

XXVI CONVEGNO DELLA SOCIETA' ITALIANA DI CHIMICA AGRARIA - Palermo, 30 settembre - 3 ottobre 2008

- What are heavy metals
- The pathway of heavy metals from the root to the shoot
- Modulating heavy metal uptake at the root level
- The role of the vacuole in heavy metal accumulation

# Modulating heavy metal contents and allocation in plants

## What is a heavy metal?

**Heavy metals are defined as those elements which have a density of more than 5 g / cm$^3$.**
**We use them for industrial products but many of them are also required for biological functions**

**Essentiel heavy metals**
Iron
Cobalt
Copper
Molybdenum
Nickel
Zink

**Not required heavy metals**
Lead
Cadmium
Arsenic

# What are the problems related to heavy metals?

**For plants:**

**Insufficient uptake of essential heavy metals**

**Uptake of two large amounts of heavy metals**

**Uptake of toxic heavy metals**

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

# What are the problems related to heavy metals?

For plants:

Insufficient uptake of essential heavy metals

Uptake of two large amounts of heavy metals

Uptake of toxic heavy metals

**For humans:**

**Insufficient amounts of essential heavy metals**

**To strong chelation of essential heavy metals**

**Contamination of soils with toxic heavy metals**

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# What are the problems related to heavy metals?

For plants:

Insufficient uptake of essential heavy metals

Uptake of two large amounts of heavy metals

Uptake of toxic heavy metals

For humans:

Insufficient amounts of essential heavy metals

To strong chelation of essential heavy metals

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

Contamination of soils with toxic heavy metals

**Approaches using breeding and plant biotechnology:**

**Safe food**

**Phytoremediation**

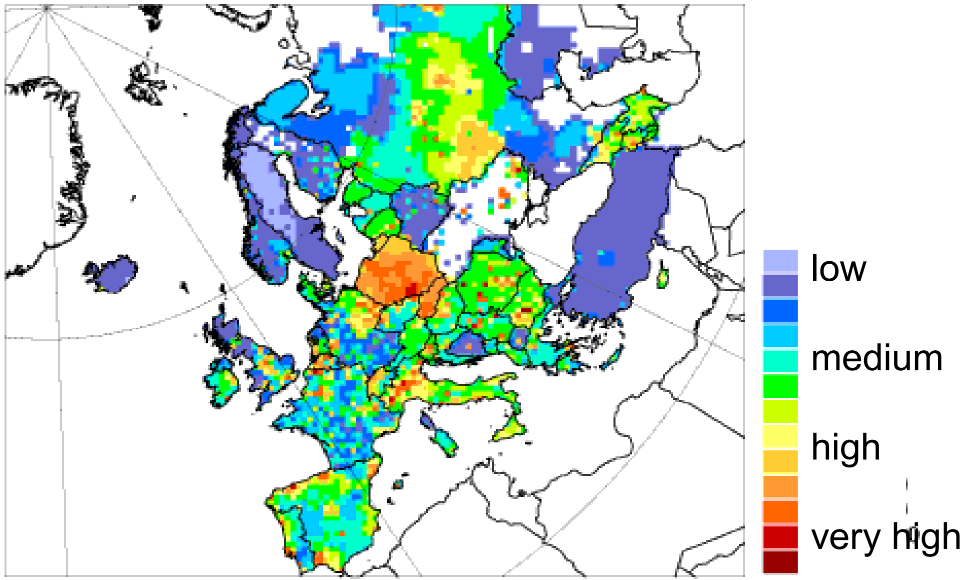
**Biofortification**

# An example for a heavy metal: Cadmium

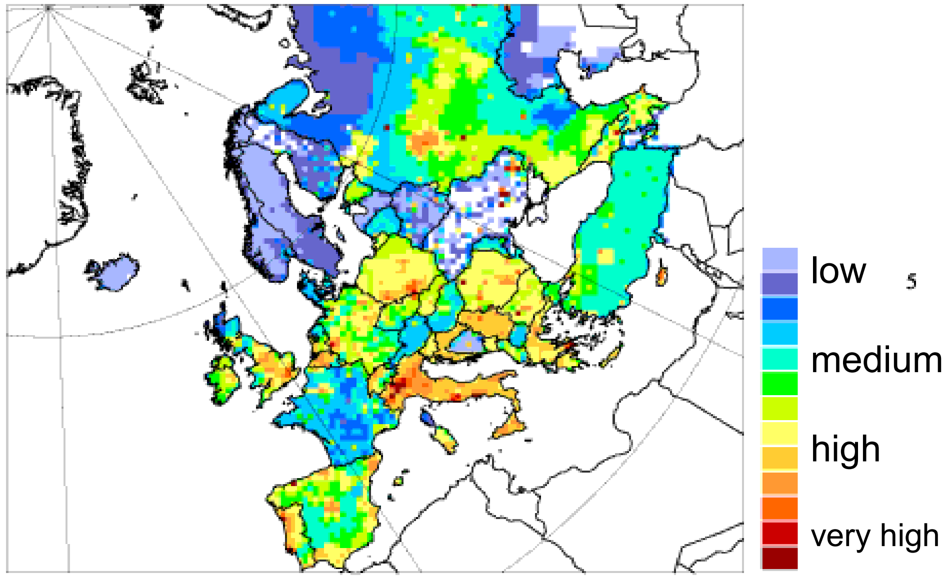
- Interacts with SH groups
- Causes oxidative stress
- In humans it damages the kidney, immunodeficiency and it is carcinogenic

# Is heavy metal contamination a problem in Europe?

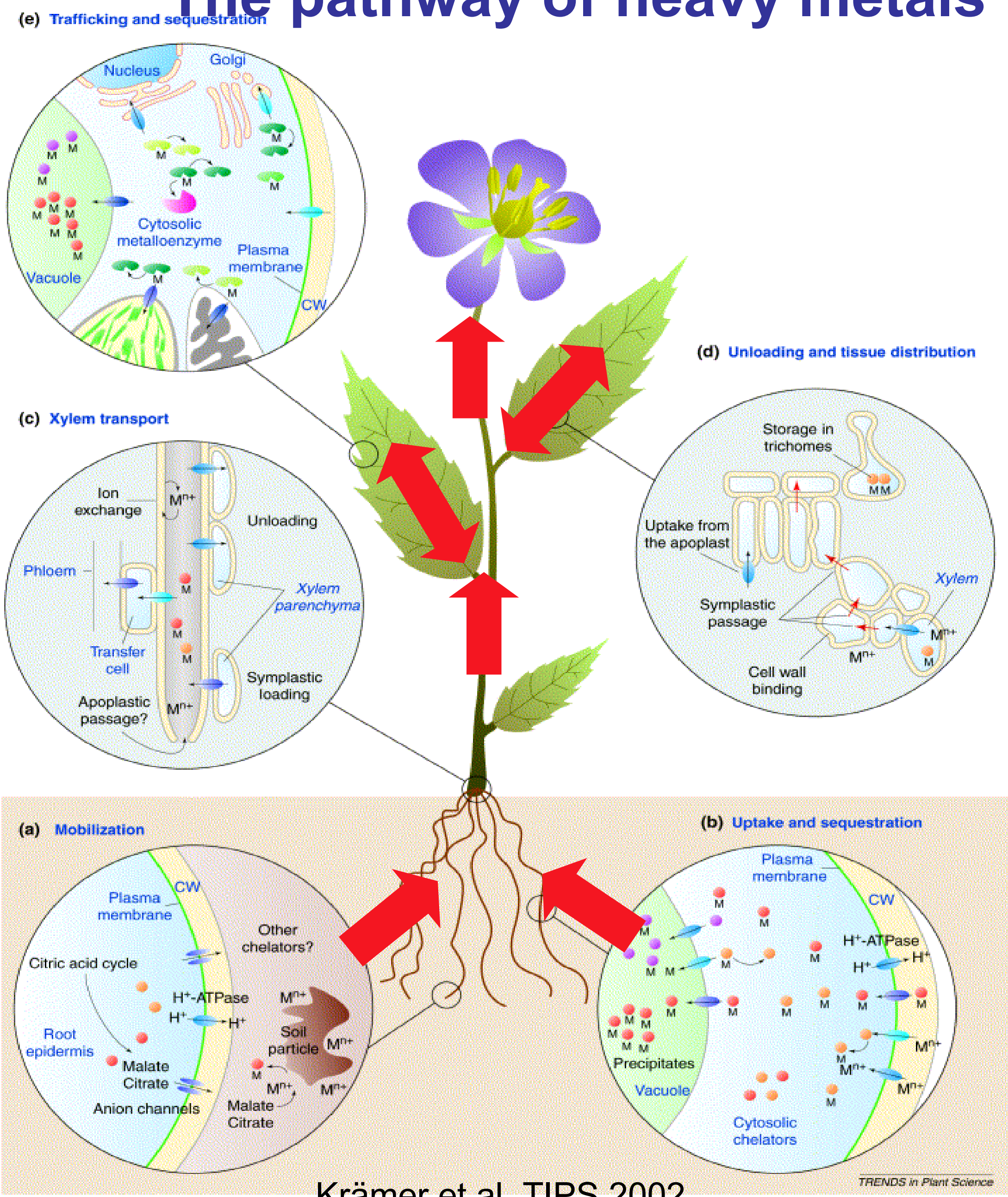
## Cadmium



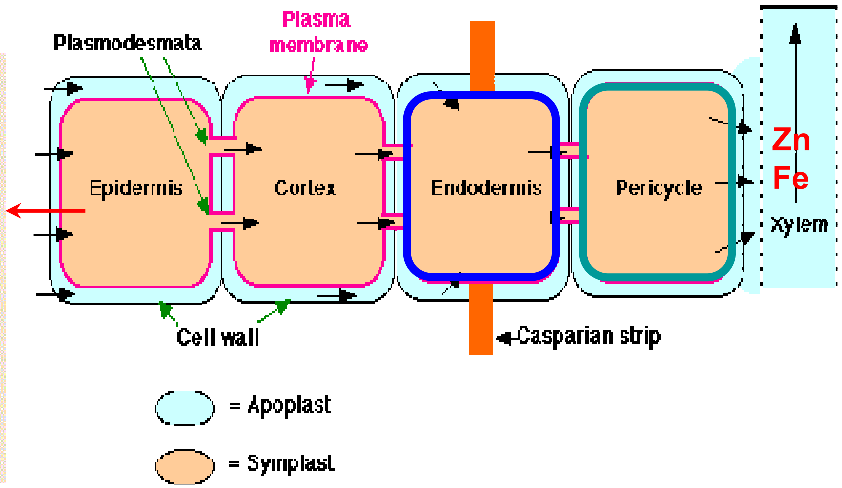

## Lead



# The pathway of heavy metals from the root to the shoot



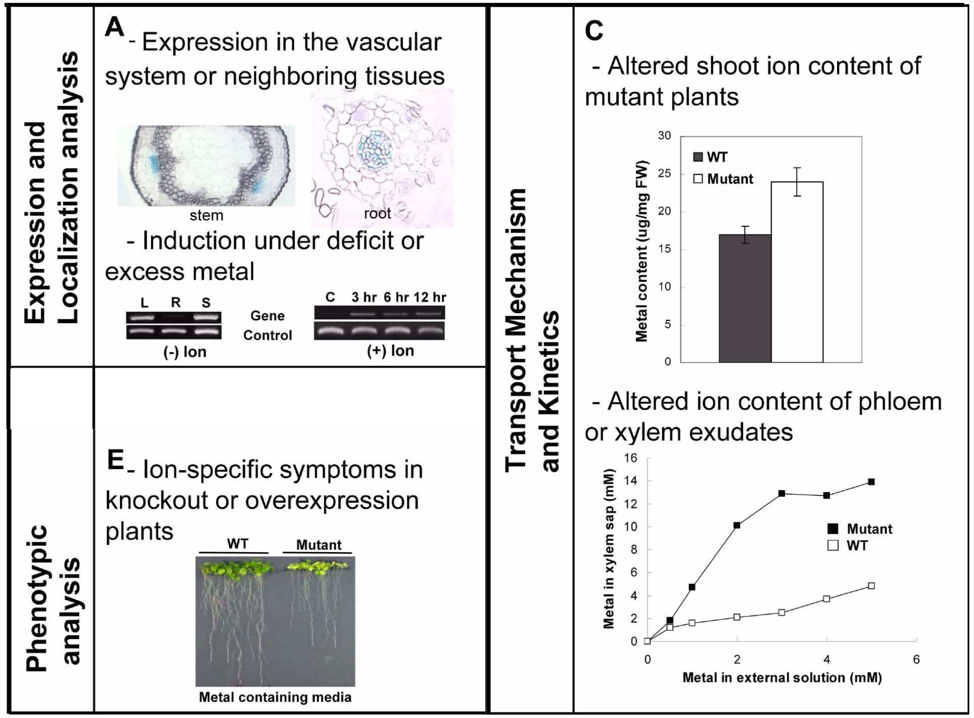

Krämer et al. TIPS 2002,

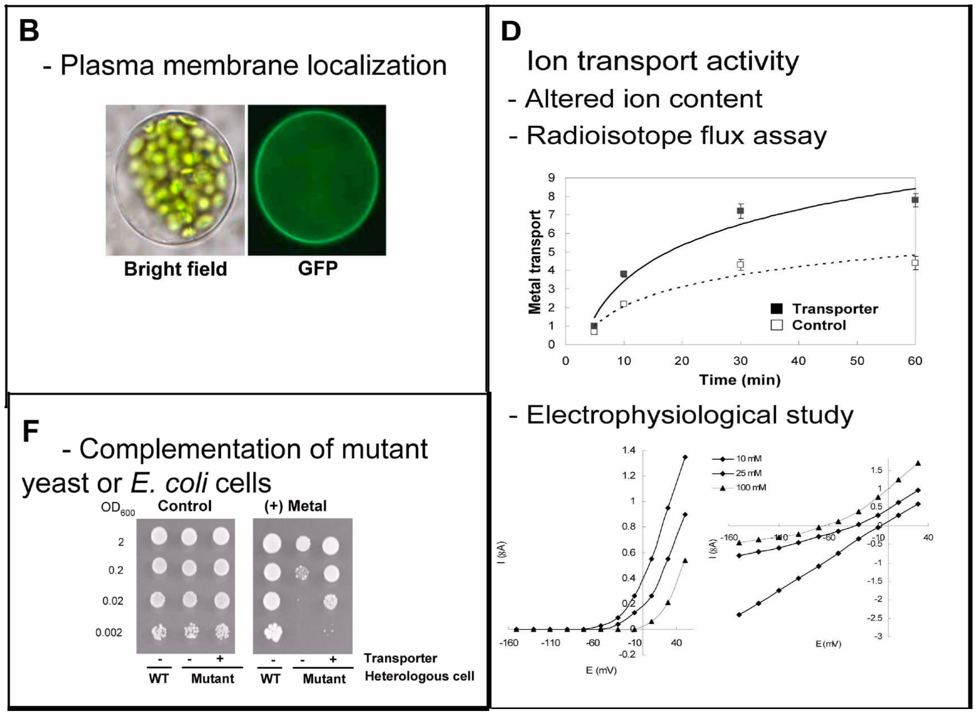


# Tools to study plant transport processes

## Whole plant level

## Single cell level

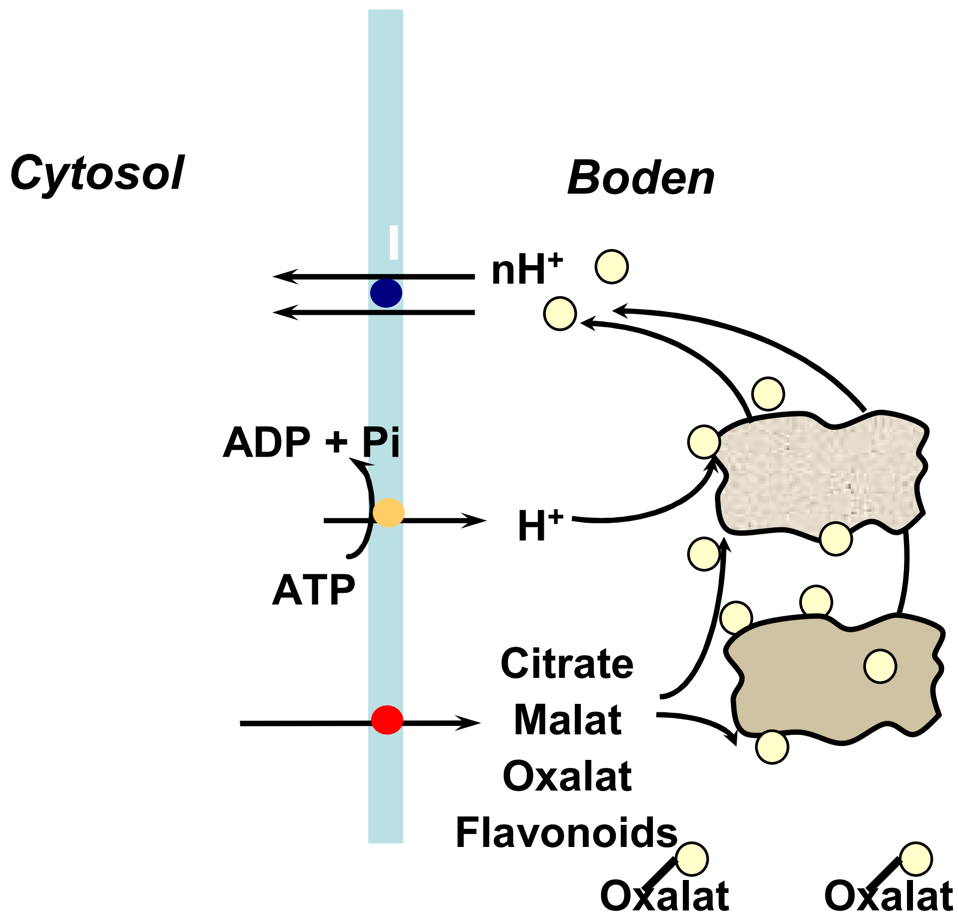



Park et al. 2008

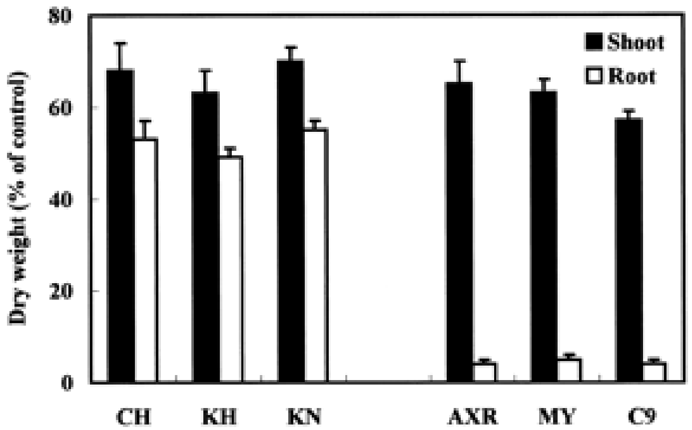
# The soil-root interaction

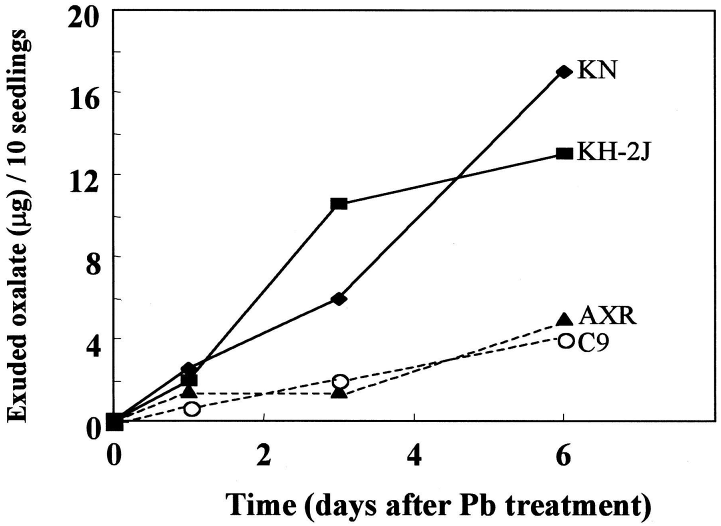
## Root exudates may either immobilze heavy metals or rend them more soluble

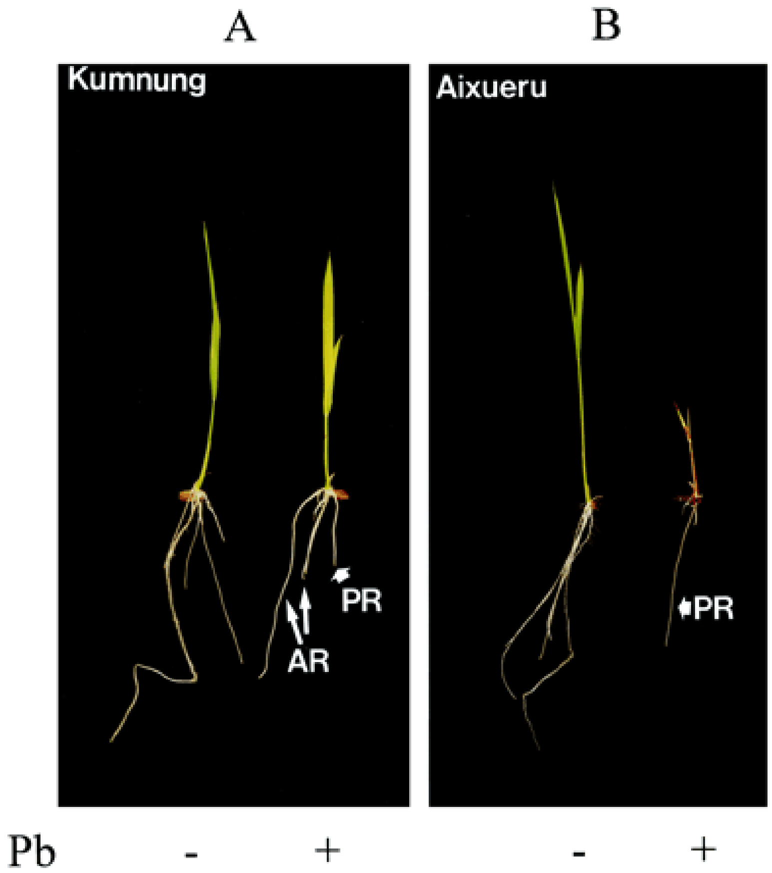


# Screening rice varieties for Pb resistance

A




Lee et al. Plant Physiol 2002

# IRT1 is the major $Fe^{2+}$ uptake transporter in roots but is also the major $Cd^{2+}$ transporter

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are needed to see this picture.

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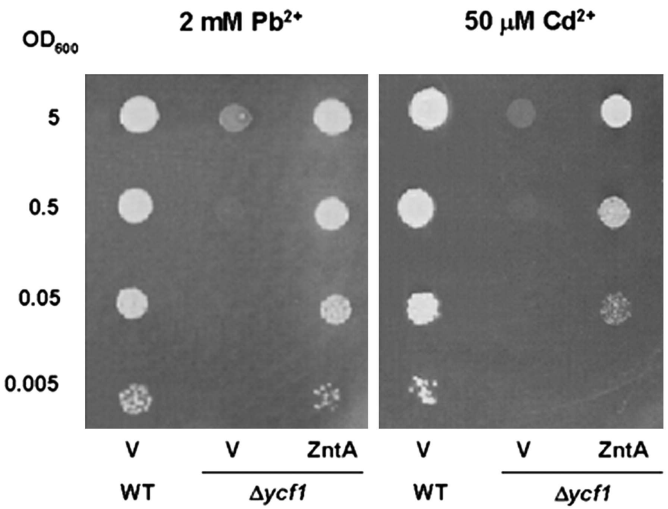
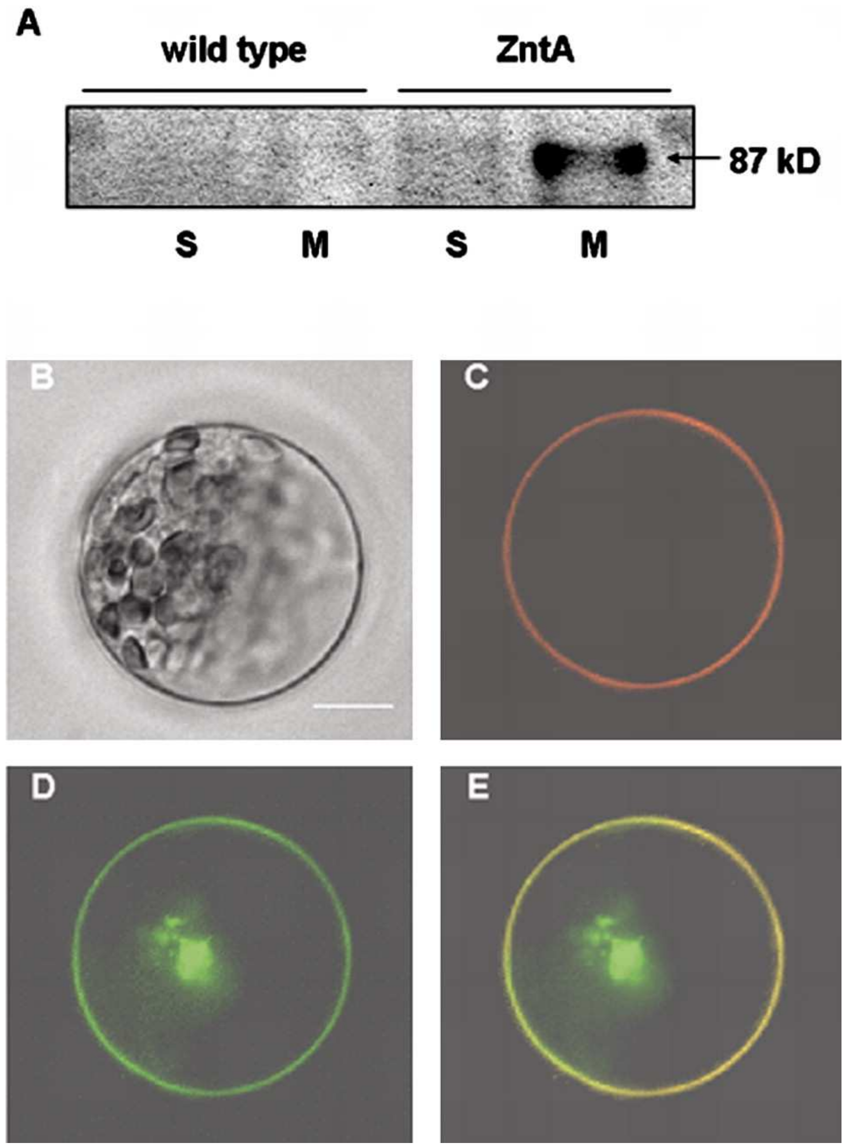
Henriques et al. 2002

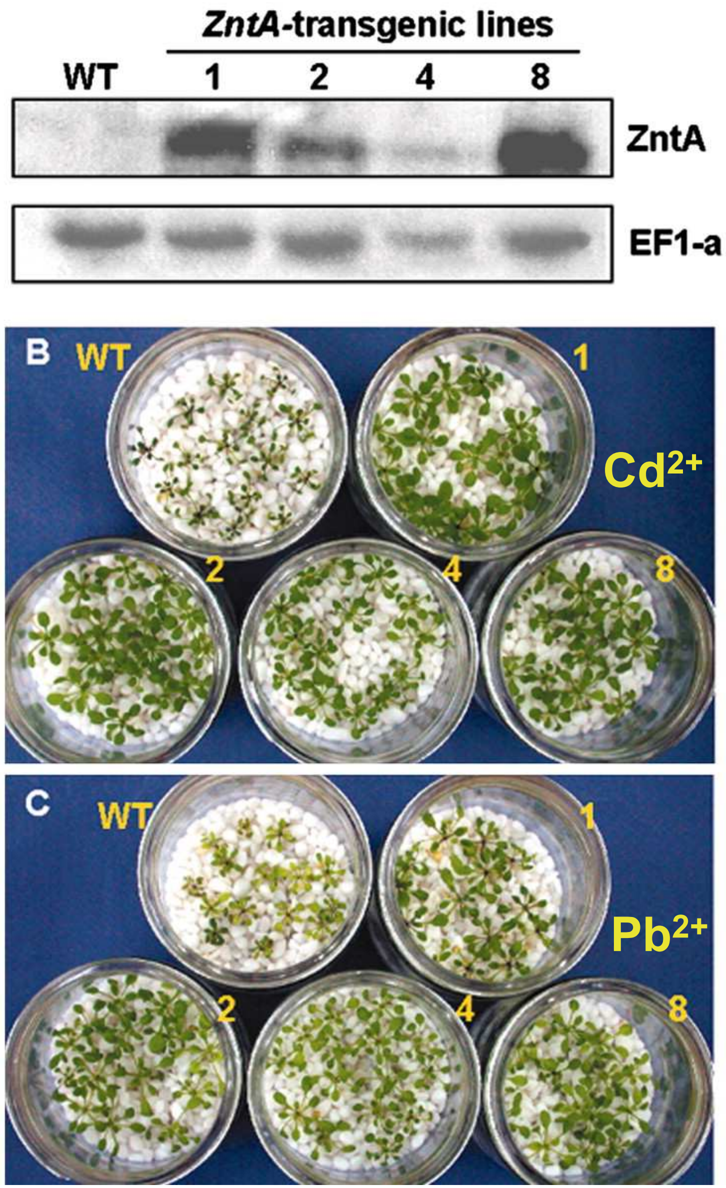
# A biotechnological approach to decrease heavy metals in plants: Expression of a bacterial cadmium transporter

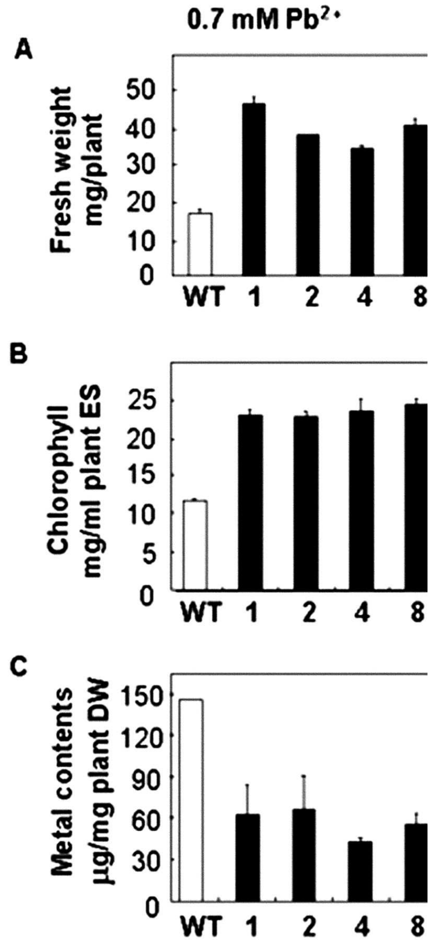


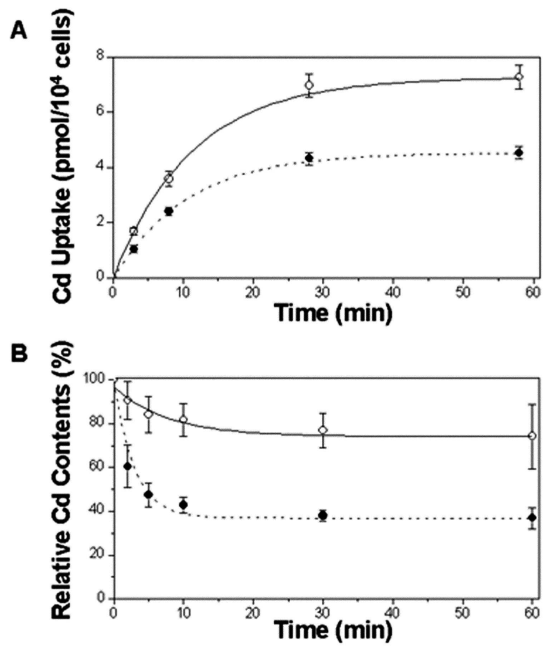




Lee et al. 2003

# Expression of a bacterial cadmium transporter to reduce cadmium uptake in plants

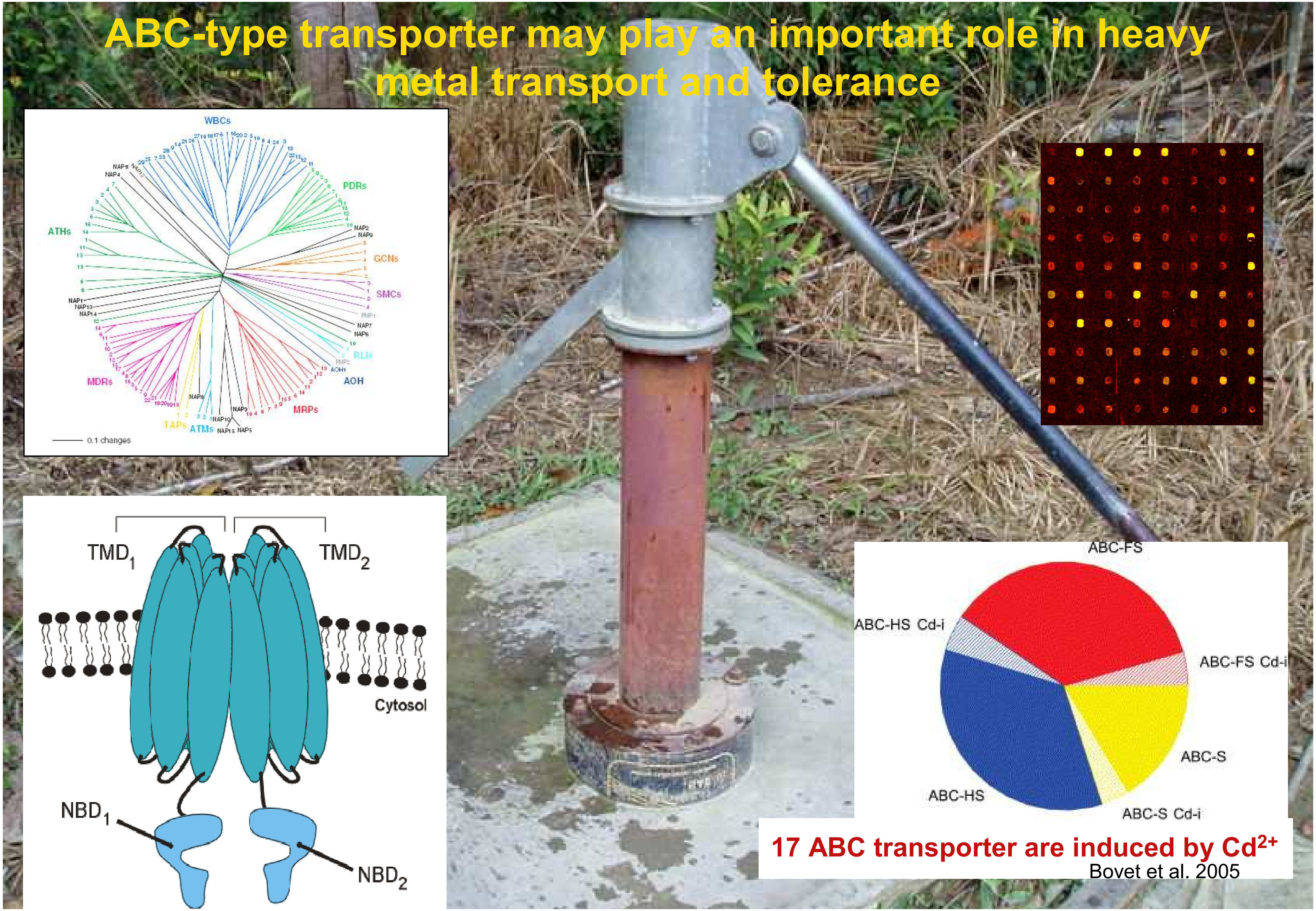



Lee et al. 2003

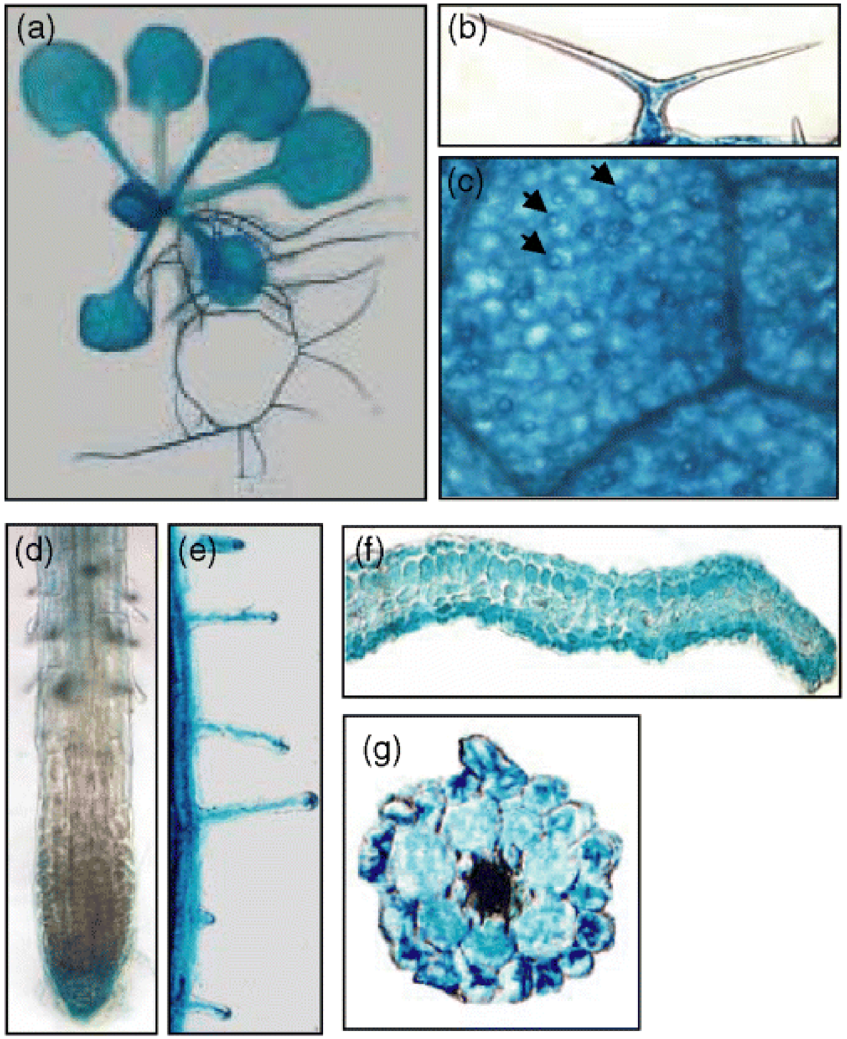
# ABC-type transporter may play an important role in heavy metal transport and tolerance

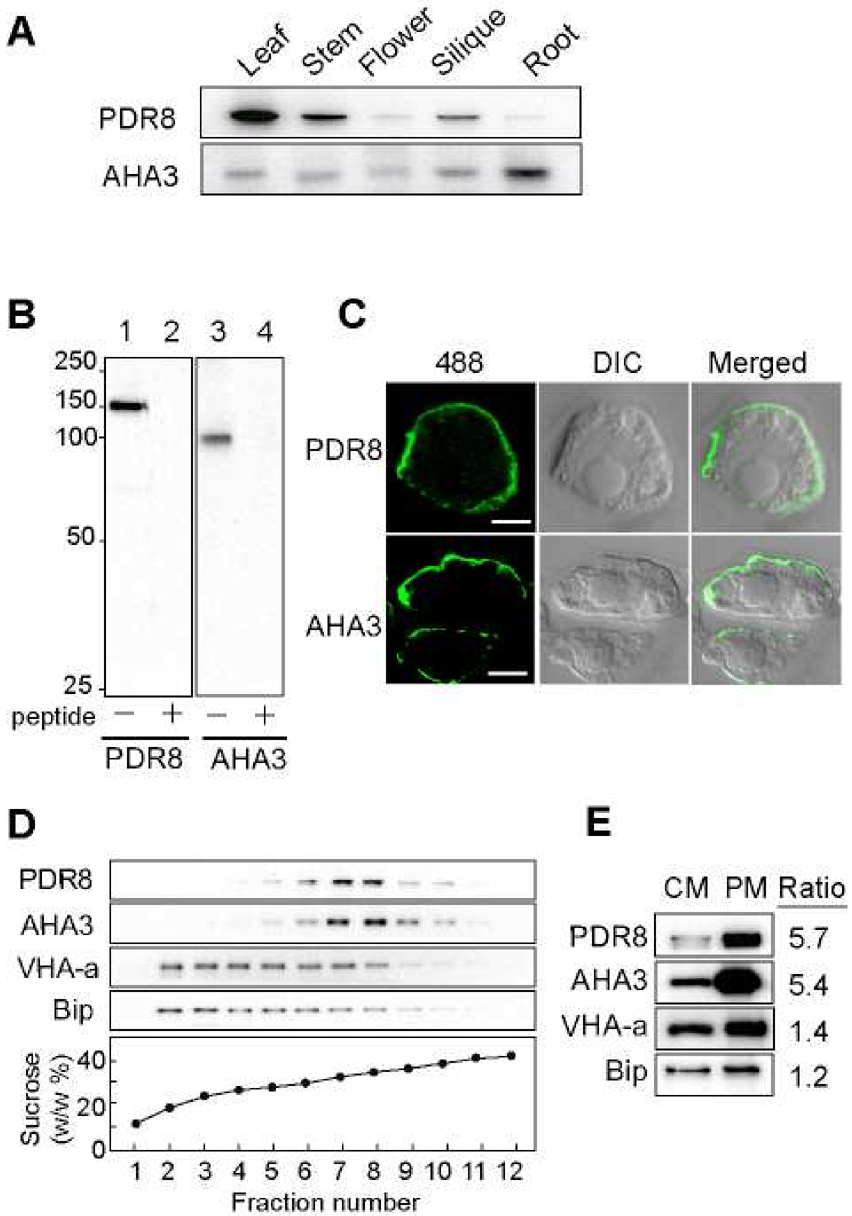



**17 ABC transporter are induced by $Cd^{2+}$**

Bovet et al. 2005

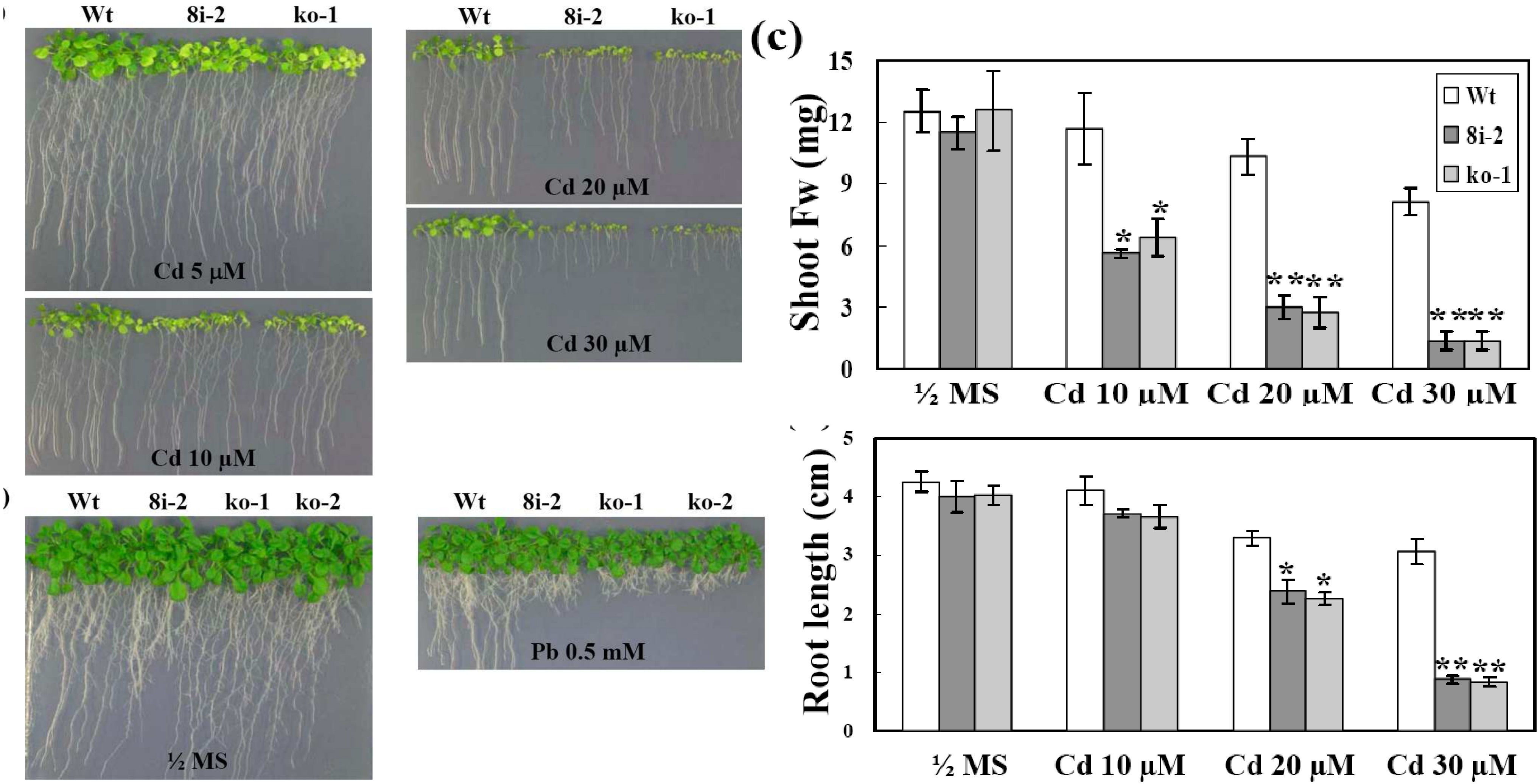
# The ABC-type transporter AtPDR8 is expressed mainly in epidermal cells and localized i the plasma membrane



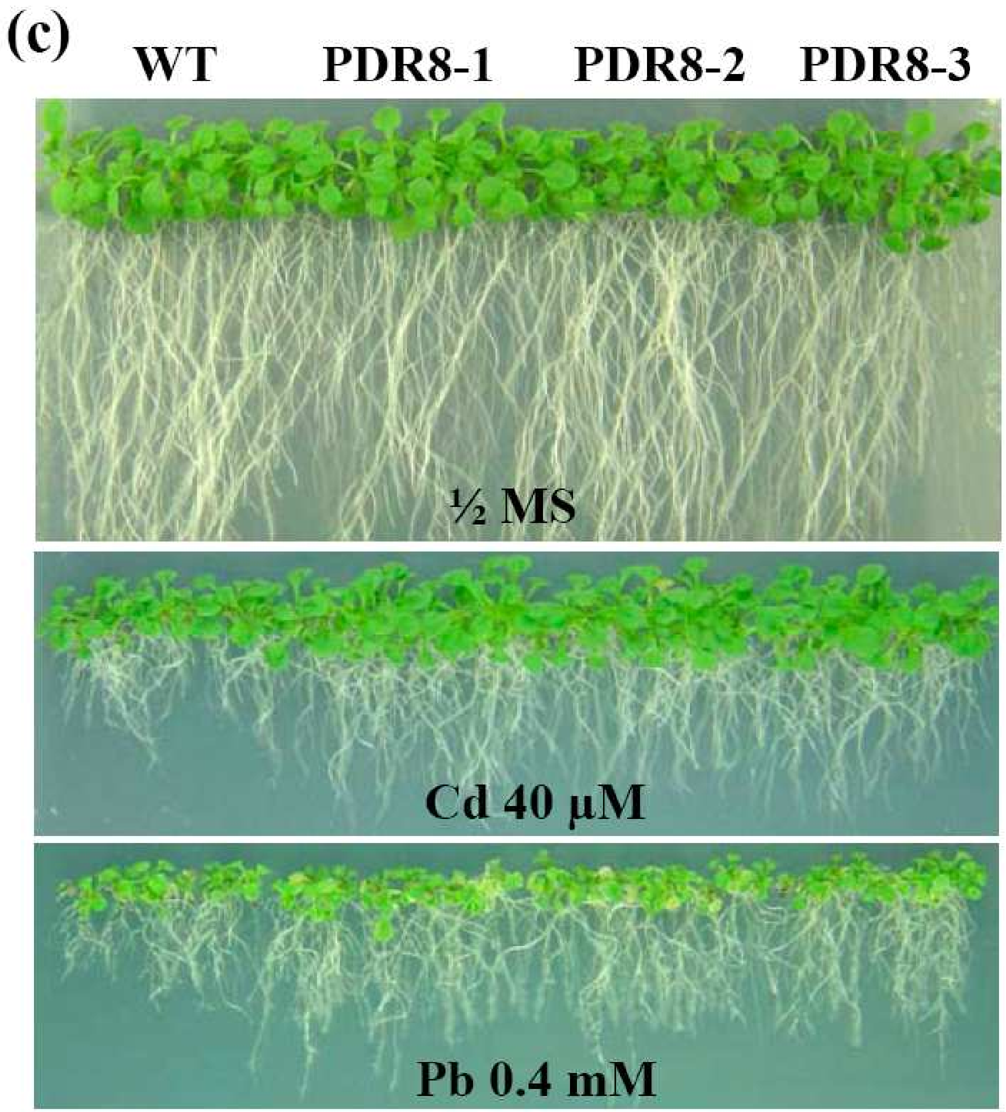

Kim, Bovet et al. Plant J. 2007

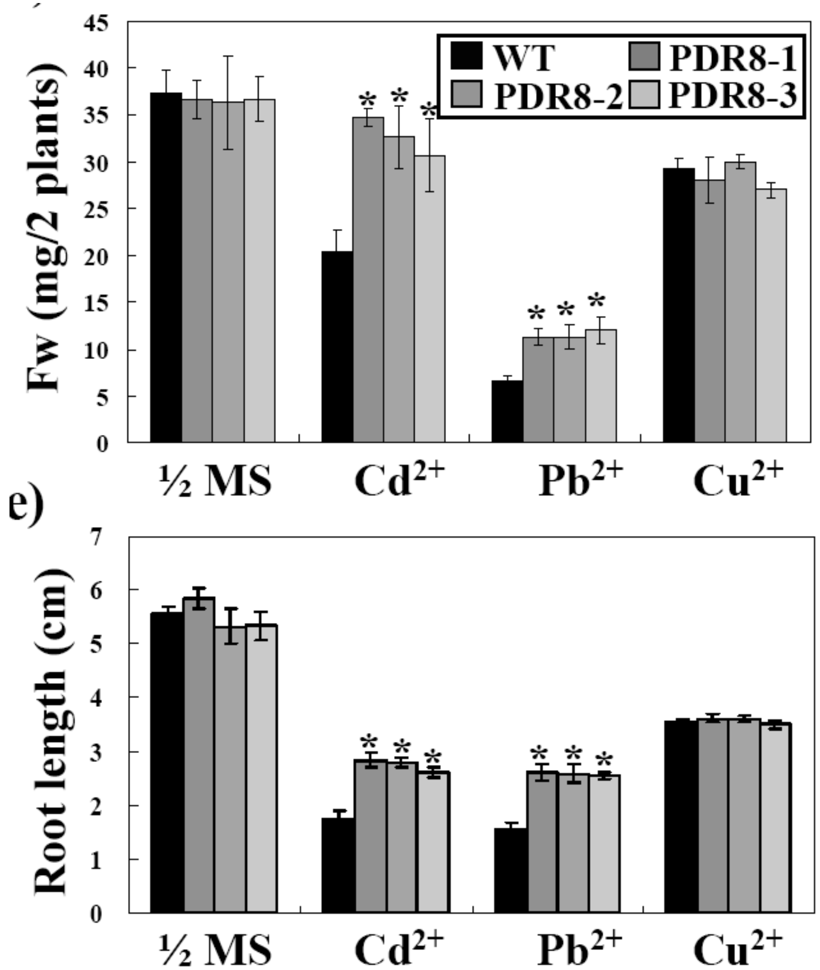
# Deletion mutants of AtPDR8 are sensitive to cadmium and lead




Kim, Bovet et al. 2007

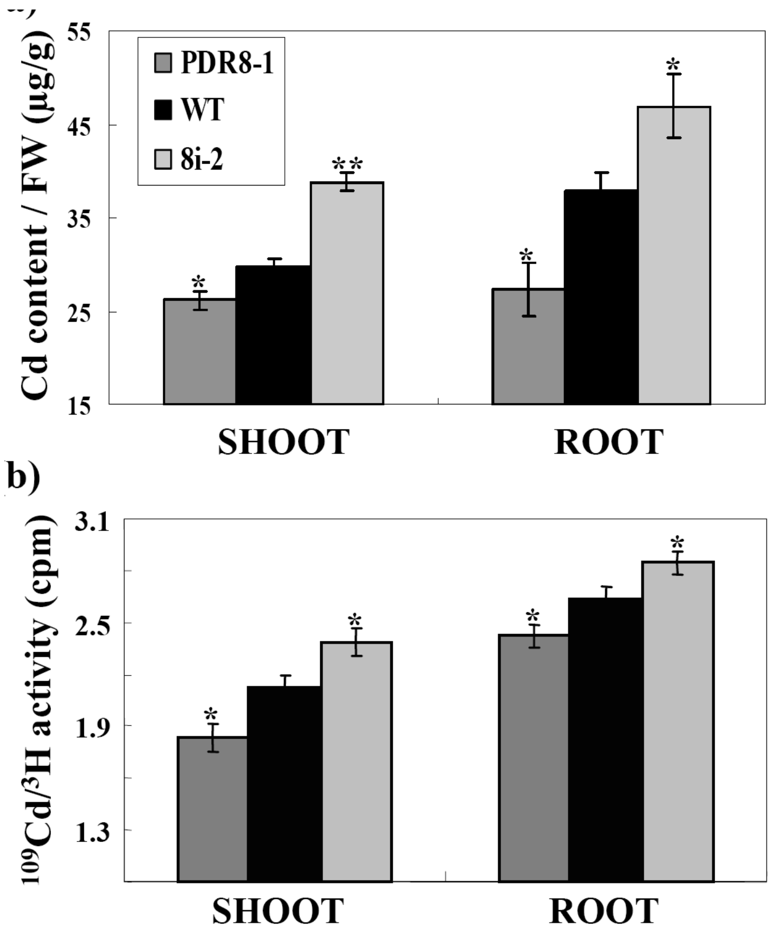
# AtPDR8 overexpressing plants are more tolerant to cadmium and lead

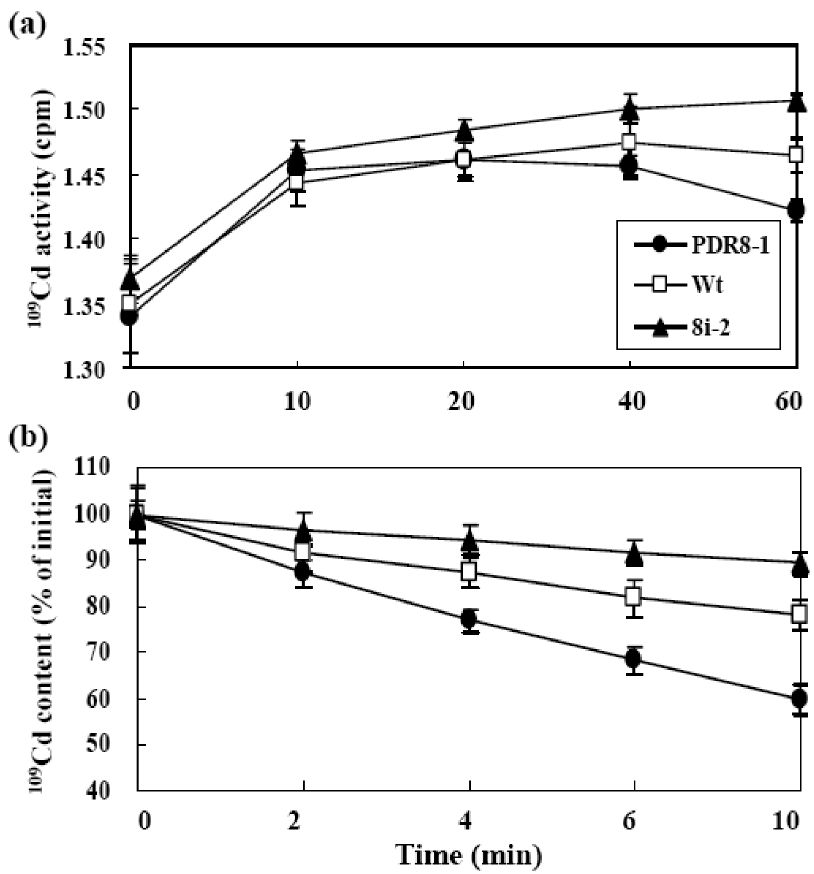



Kim, Bovet et al. 2007

# AtPDR8 acts as a cadmium efflux pump




Kim, Bovet et al. 2007

# AtPDR8 is involved in heavy metal and biotic stress: Where is the link?

***Rapid Paper***

**Loss of AtPDR8, a Plasma Membrane ABC Transporter of *Arabidopsis thaliana*, Causes Hypersensitive Cell Death Upon Pathogen Infection**

**Yoshihiro Kobae [1], Tetsuro Sekino [1], Hirofumi Yoshioka [2], Tsuyoshi Nakagawa [3], Enrico Martinoia [4] and Masayoshi Maeshima [1, *]**

The Plant Cell, Vol. 18, 731–746, March 2006, www.plantcell.org © 2006 American Society of Plant Biologists

**_Arabidopsis_ PEN3/PDR8, an ATP Binding Cassette Transporter, Contributes to Nonhost Resistance to Inappropriate Pathogens That Enter by Direct Penetration** W OA

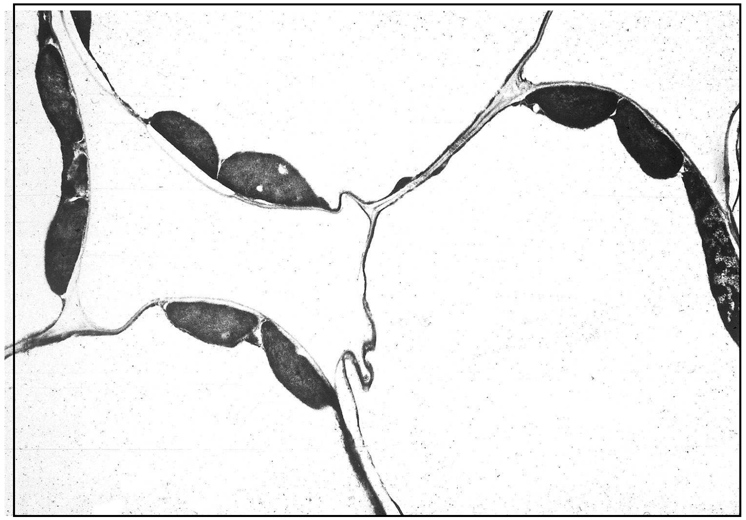
**Mónica Stein,[a] Jan Dittgen,[b] Clara Sánchez-Rodríguez,[c] Bi-Huei Hou,[a] Antonio Molina,[c] Paul Schulze-Lefert,[b] Volker Lipka,[d] and Shauna Somerville[a,1]**

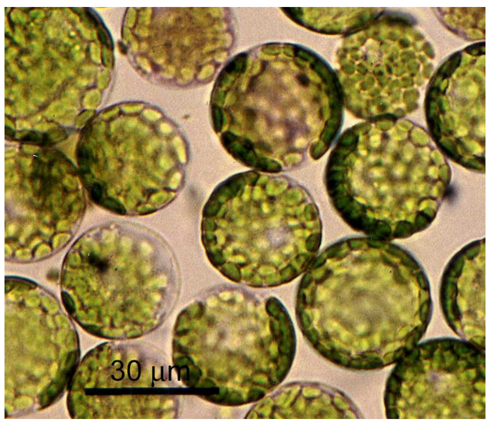
*The Plant Journal* (2007) **50**, 207–218 doi: 10.1111/j.1365-313X.2007.03044.x

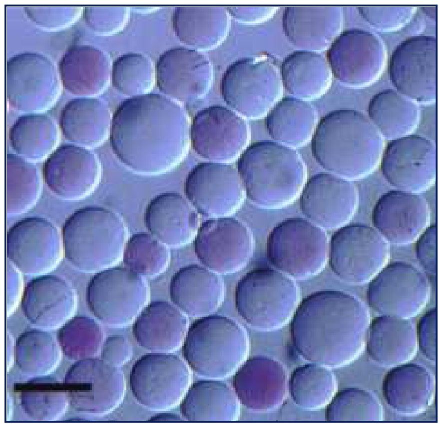
**The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance**

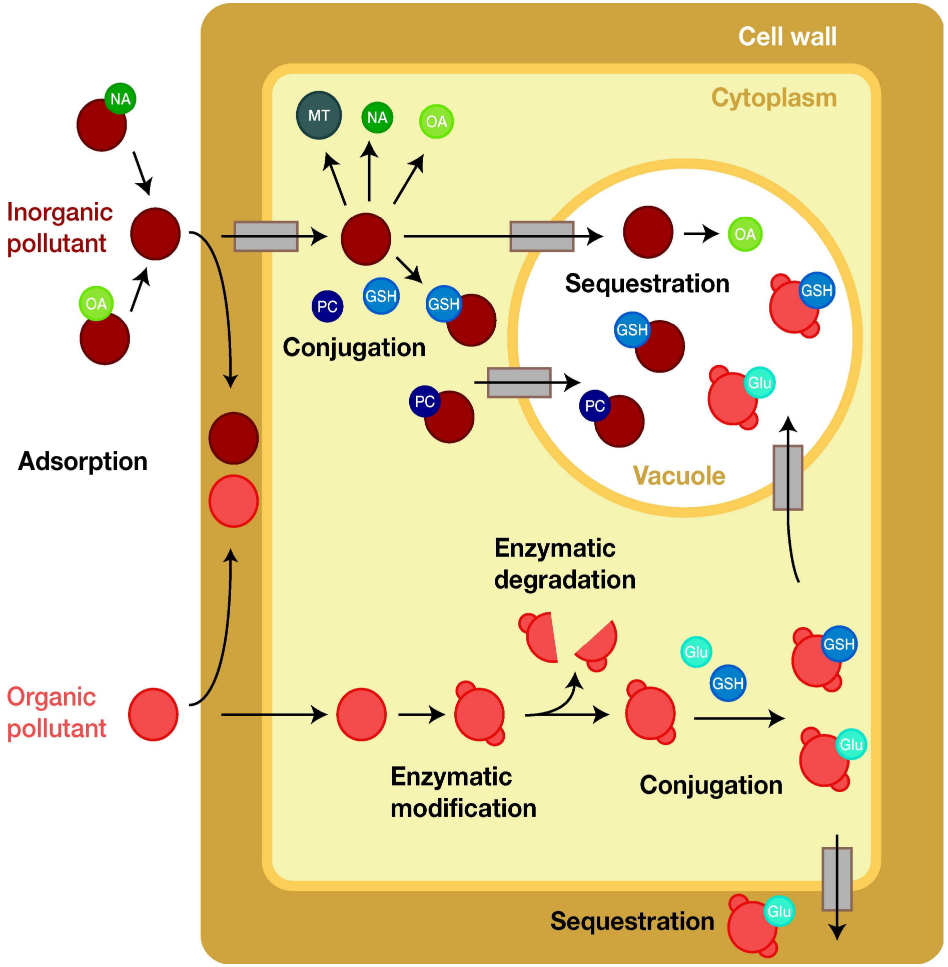
**Do-Young Kim[1], Lucien Bovet[2], Masayoshi Maeshima[3], Enrico Martinoia[1,4,†] and Youngsook Lee[1,*,†]**

# Exploring the role of the vacuole to increase heavy metal allocation and accumulation

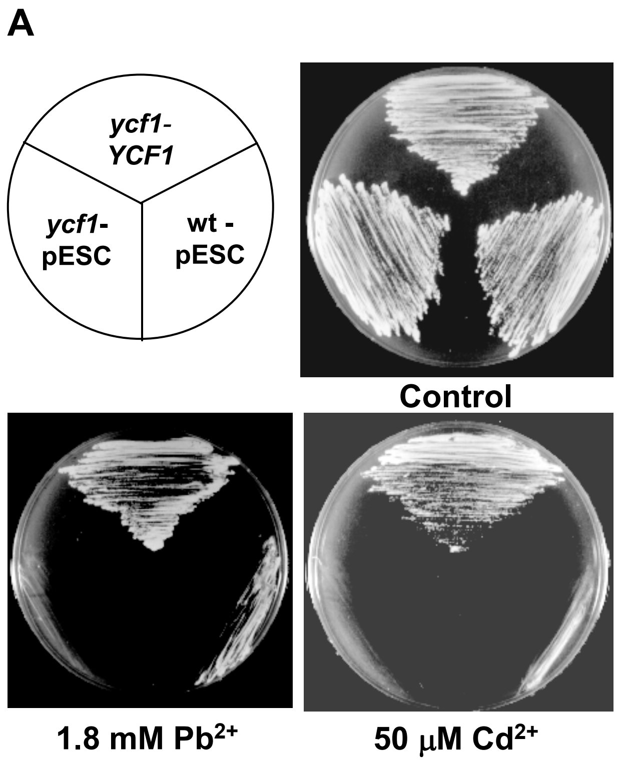


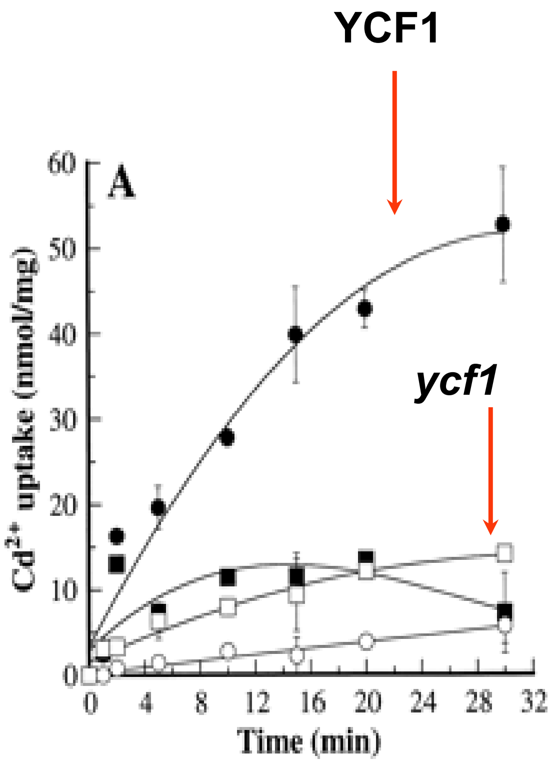




# Is vacuolar transport a limiting factor for heavy metal tolerance? Expression of the yeast bis-GS-Cd transporter YCF1 in plants




Song et al. Nature Biotech. 2003

# Arabidopsis expressing YCF1 exhibit a higher $GS_2$-Cd transport

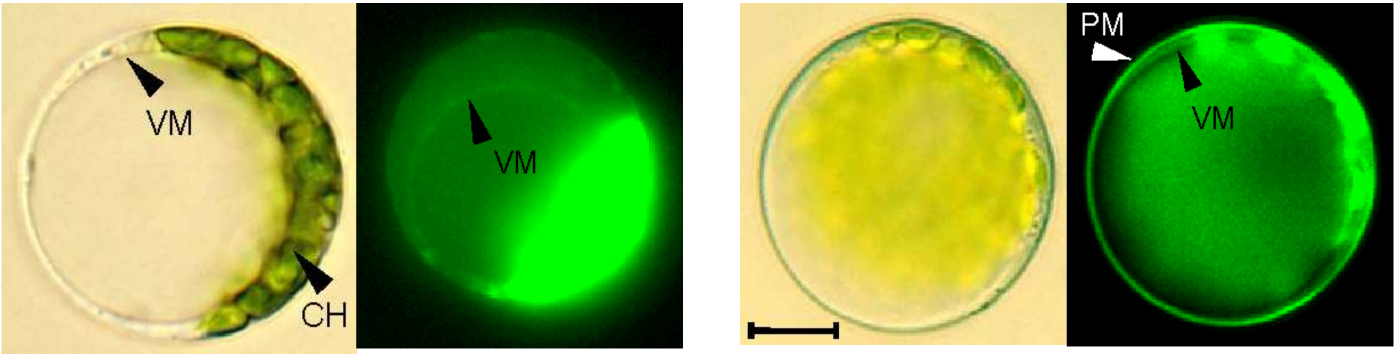



**Table 1 Uptake of GS-conjugated cadmium and reduced glutathione into intact vacuoles isolated from wild-type and *YCF1*-transgenic plants**

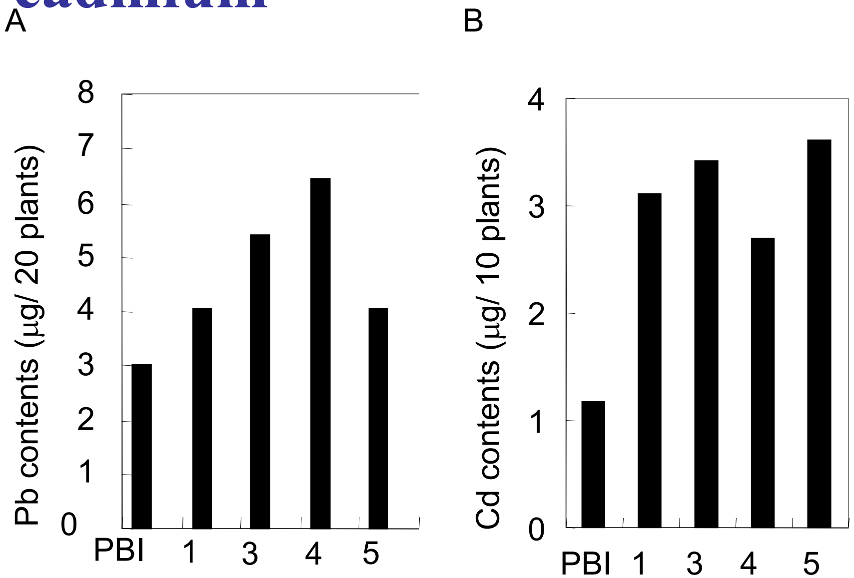
| Compound | Wild-type plants, nmol/ml vacuole/min | *YCF1*-transgenic plants, nmol/ml vacuole/min |
|---|---|---|
| Cd + GSH | 1.45 ± 0.25 | 5.30 ± 0.49 |
| GSH | 0.15 ± 0.11 | 0.20 ± 0.06 |

Substrates tested were 200 μM GSH containing $^3H$-labeled GSH with or without 200 μM $CdCl_2$. Values shown are means ± s.e.m. ($n$ = 10) from two independent experiments, each with five replicates.

Song et al. Nature Biotech. 2003

# Arabidopsis expressing YCF1 are more tolerant to arsenic, lead and cadmium


**1/2 MS + 50 mM As(V)**

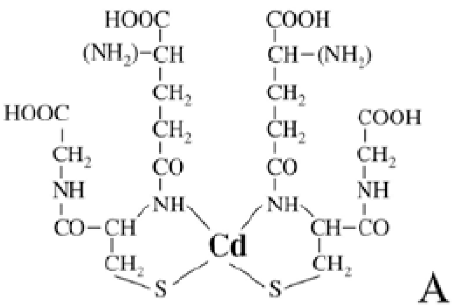


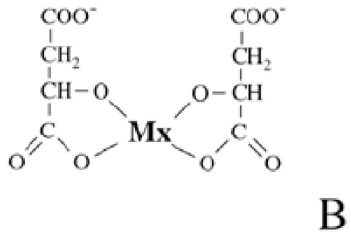


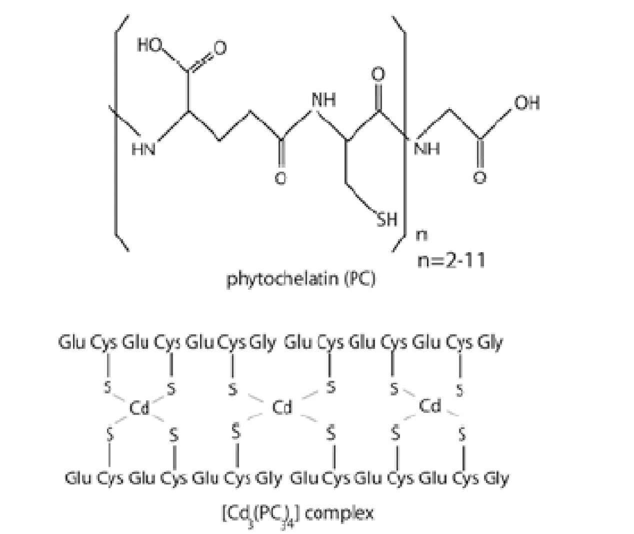
**1/2 MS + 1 mM $Pb^{2+}$**

Song et al. Nature Biotech. 2003

# Can the vacuolar sink be increased byby increasing the amount of chelators?




**Glutathion-Cd** **Malat-Mx** **Phytochelatin**

**Malate is a central metabolite in plants:**

Metabolic pathways: Kebs cycle, glyoxylate cycle, $CO_2$ fixation, malate decarboxylation

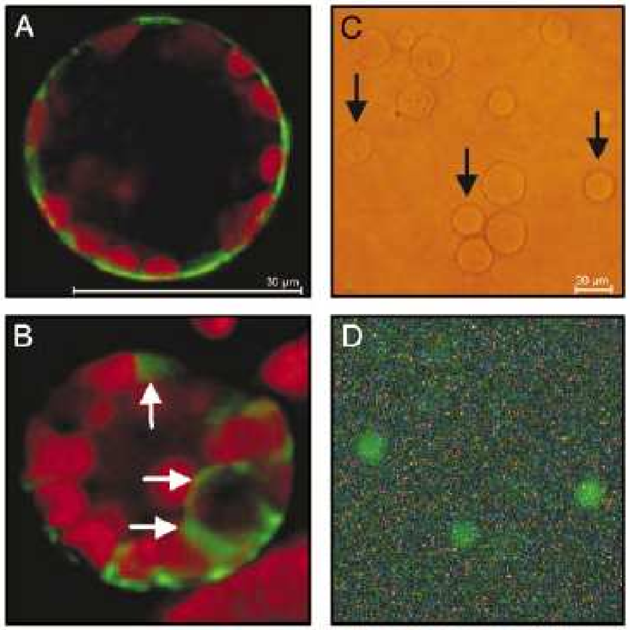
Store for $CO_2$ and reduction equivalents

Important osmolyte (stomata, CAM, C4)

pH state

Chelator (intracellular (mainly zinc) and extrcellular (aluminum tolerance)

# A homologue of the renal Na+/dicarboxylate transporter is the vacuolar malate transporter

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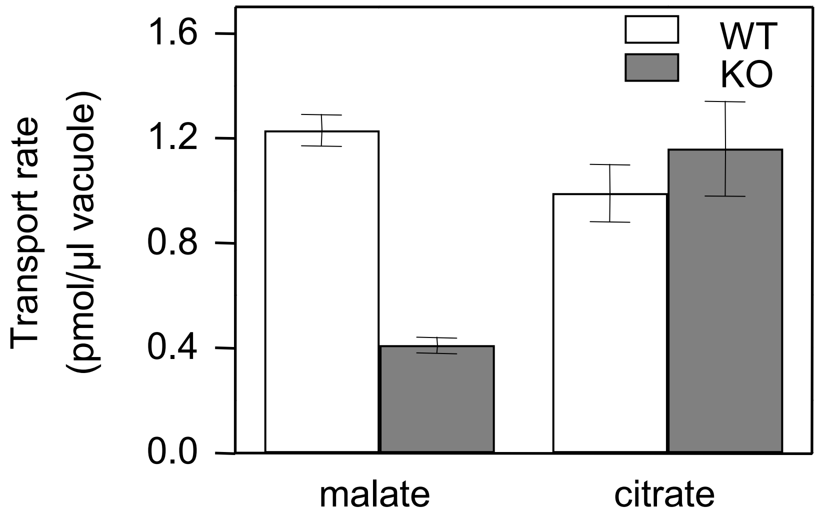


Emmerlich et al. 2003

# AttDT is the vacuolar malate transporter, but not the the malate channel

## Current densities in wt and attdt KO are similar

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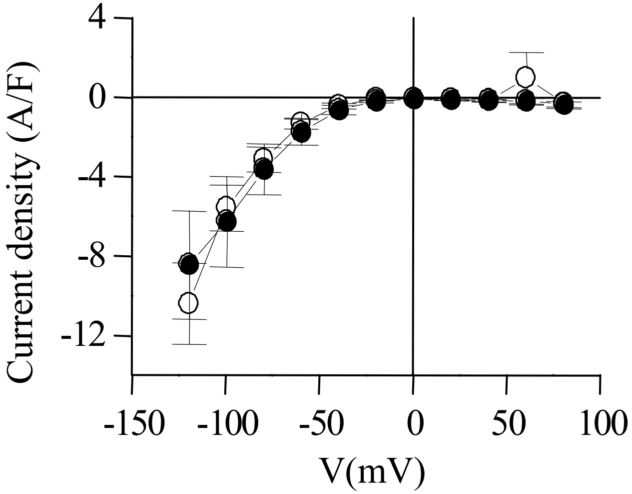
TIFF (Uncompressed) decompressor
are needed to see this picture.

b




## The currents detected are due to malate

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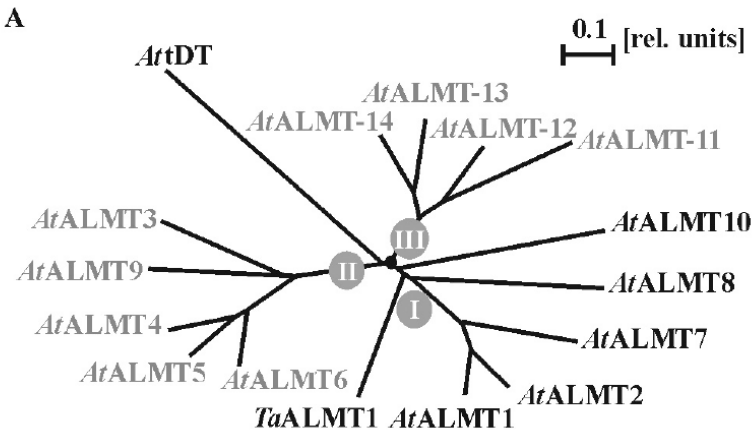
are needed to see this picture.

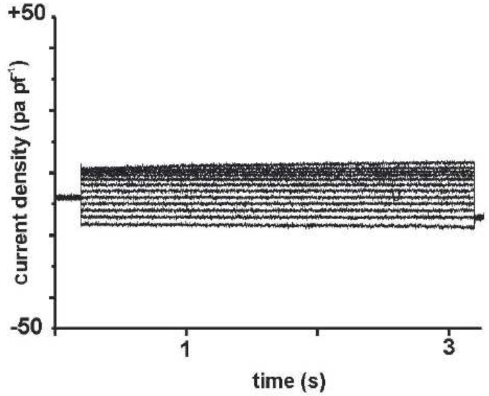
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Hurth, Suh et al. 2005

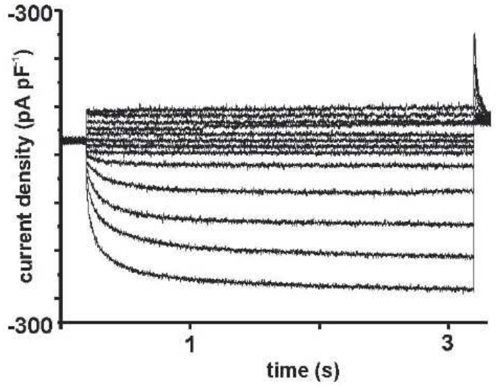
# The vacuolar malate channels are members of the ALMT family




a)




b)




QuickTime™ and a
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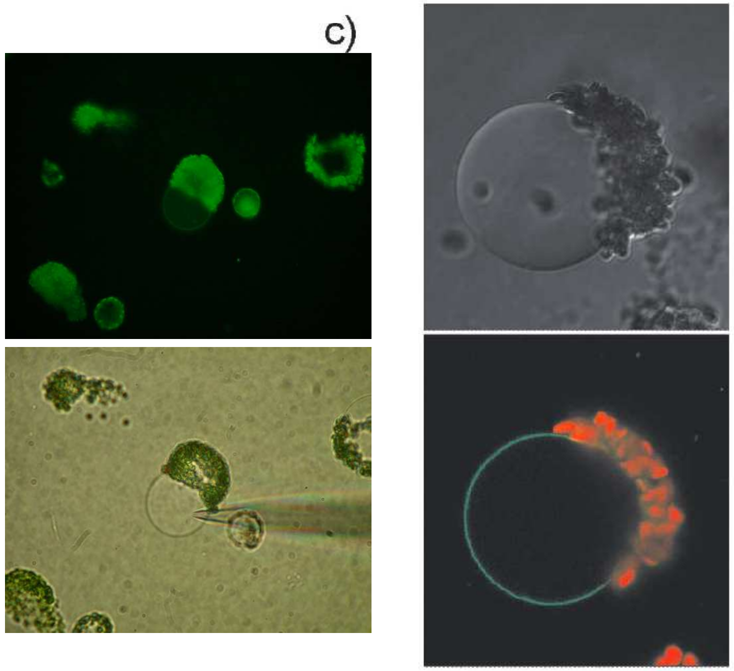
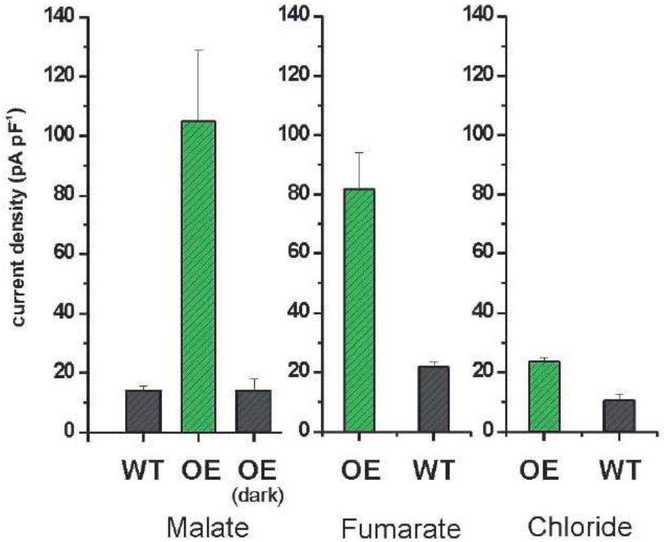
c)

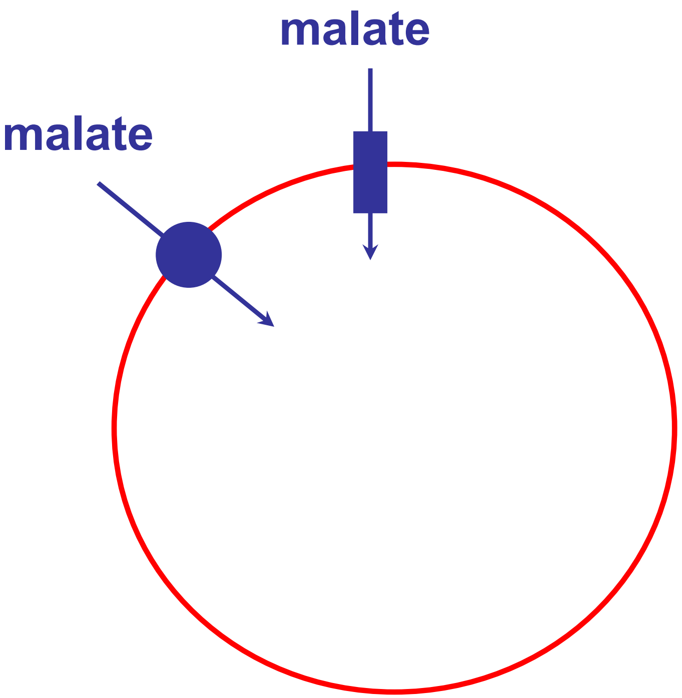


d)




Kovermann, Meyer et al., Plant J. 2007

# Impairing vacuolar malate transport has no effect on heavy metal resistance




| ENTRY | NAME | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 17 | a-tocopherol | 0.957 | 1.00 | ± | 0.2662 | 7.597 | 0.1036 | 7.938 | ± | 0.495 |
| 21 | maltitol | 0.814 | 1.00 | ± | 0.1757 | 0.7994 | 0.9447 | 0.982 | ± | 0.196 |
| 23 | maltose MX2 | 0.563 | 1.00 | ± | 0.0474 | 0.6008 | 0.6362 | 1.068 | ± | 0.152 |
| 25 | trehalose | 0.446 | 1.00 | ± | 0.057 | 0.5429 | 0.0323 | **1.219** | ± | **0.058** |
| 27 | trehalose | 0.830 | 1.00 | ± | 0.070 | 1.0275 | 0.0538 | 1.238 | ± | 0.072 |
| 29 | maltose MX1 | 0.443 | 1.00 | ± | 0.1082 | 0.6772 | 0.5822 | 1.53 | ± | 0.605 |
| 31 | sucrose | 11.85 | 1.00 | ± | 0.0688 | 13.654 | 0.1013 | 1.152 | ± | 0.041 |
| 47 | 18:0 | 9.461 | 1.00 | ± | 0.0734 | 8.557 | 0.2749 | 0.904 | ± | 0.034 |
| 59 | myo-ino | 8.315 | 1.00 | ± | 0.0353 | 12.792 | 0.0217 | **1.539** | ± | **0.142** |
| 63 | 16:0 | 5.326 | 1.00 | ± | 0.0297 | 5.0926 | 0.3572 | 0.956 | ± | 0.037 |
| 69 | gluconic | 0.133 | 1.00 | ± | 0.0641 | 0.1213 | 0.3918 | 0.915 | ± | 0.079 |
| 77 | D-isoascorbic | 0.227 | 1.00 | ± | 0.0614 | 0.2537 | 0.2486 | 1.118 | ± | 0.069 |
| 81 | L-ascorbic | 0.227 | 1.00 | ± | 0.0612 | 0.2537 | 0.244 | 1.119 | ± | 0.069 |
| 87 | sorbitol/galactitol | 0.214 | 1.00 | ± | 0.3011 | 0.1319 | 0.2713 | 0.615 | ± | 0.153 |
| 89 | mannitol | 0.218 | 1.00 | ± | 0.293 | 0.1319 | 0.2459 | 0.604 | ± | 0.153 |
| 97 | glc MX1 | 16.35 | 1.00 | ± | 0.0998 | 28.244 | 0.001 | **1.728** | ± | **0.084** |
| 99 | gal MX1 | 0.144 | 1.00 | ± | 0.0428 | 0.159 | 0.0703 | 1.104 | ± | 0.025 |
| 101 | man MX | 0.073 | 1.00 | ± | 0.0652 | 0.0855 | 0.0835 | 1.17 | ± | 0.053 |
| 103 | fru MX2 | 1.825 | 1.00 | ± | 0.1094 | 4.0514 | 0.0099 | **2.22** | ± | **0.187** |
| 105 | fru MX1 | 2.475 | 1.00 | ± | 0.1137 | 5.6209 | 0.0102 | **2.271** | ± | **0.192** |
| 107 | quinic | 1.269 | 1.00 | ± | 0.26 | 4.8436 | 0.2234 | 3.817 | ± | 0.615 |
| 109 | dehydroascorbic | 0.927 | 1.00 | ± | 0.0239 | 1.0258 | 0.0189 | **1.107** | ± | **0.03** |
| 111 | dehydroascorbic | 2.238 | 1.00 | ± | 0.0233 | 2.4977 | 0.0167 | **1.116** | ± | **0.033** |
| 119 | citric | 2.381 | 1.00 | ± | 0.1409 | 3.8215 | 0.0105 | **1.605** | ± | **0.09** |
| 123 | shikimic | 4.489 | 1.00 | ± | 0.1628 | 13.142 | 0.2107 | 2.927 | ± | 0.534 |
| 125 | shikimic | 0.119 | 1.00 | ± | 0.1715 | 0.3728 | 0.2112 | 3.14 | ± | 0.553 |
| 155 | rhamnose MX1 | 0.249 | 1.00 | ± | 0.0867 | 0.3609 | 0.0304 | **1.448** | ± | **0.118** |
| 157 | ribose MX | 0.065 | 1.00 | ± | 0.0369 | 0.0751 | 0.0129 | **1.156** | ± | **0.034** |
| 161 | arabinose MX | 0.215 | 1.00 | ± | 0.0483 | 0.2204 | 0.6501 | 1.026 | ± | 0.023 |
| 163 | arabinose MX | 0.103 | 1.00 | ± | 0.04 | 0.1146 | 0.0639 | 1.109 | ± | 0.031 |
| 171 | xylose MX1 | 0.046 | 1.00 | ± | 0.0481 | 0.0501 | 0.2674 | 1.08 | ± | 0.046 |
| 181 | threonic | 0.808 | 1.00 | ± | 0.0469 | 0.9508 | 0.0133 | 1.177 | ± | 0.033 |
| 189 | pyroglutamic | 35.41 | 1.00 | ± | 0.0982 | 34.288 | 0.8029 | 0.968 | ± | 0.073 |
| 191 | aspartic 3 | 0.59 | 1.00 | ± | 0.2711 | 0.4663 | 0.5602 | 0.79 | ± | 0.19 |
| 203 | malic | 2.356 | 1.00 | ± | 0.0329 | 1.5738 | 0.0021 | **0.668** | ± | **0.133** |
| 205 | citramalic | 0.085 | 1.00 | ± | 0.041 | 0.046 | 7E-07 | **0.544** | ± | **0.051** |
| 207 | homoserine 3 | 0.46 | 1.00 | ± | 0.1564 | 0.5106 | 0.8368 | 1.111 | ± | 0.45 |
| 223 | threonine 3 | 1.229 | 1.00 | ± | 0.1097 | 1.5553 | 0.2251 | 1.265 | ± | 0.146 |
| 225 | serine 3 | 4.646 | 1.00 | ± | 0.0593 | 4.6528 | 0.9923 | 1.001 | ± | 0.137 |
| 227 | fumaric | 84.38 | 1.00 | ± | 0.0221 | 81.684 | 0.4944 | 0.968 | ± | 0.043 |
| 231 | glyceric | 1.23 | 1.00 | ± | 0.0578 | 1.2903 | 0.5034 | 1.049 | ± | 0.036 |
| 235 | succinic | 2.885 | 1.00 | ± | 0.0219 | 1.6026 | 6E-10 | **0.555** | ± | **0.028** |
| 239 | glycine 3 | 18.28 | 1.00 | ± | 0.1164 | 39.56 | 0.0235 | **2.164** | ± | **0.217** |
| 245 | proline 2 | 0.679 | 1.00 | ± | 0.0734 | 1.0569 | 0.0192 | **1.557** | ± | **0.133** |
| 247 | isoleucine 2 | 0.434 | 1.00 | ± | 0.1115 | 0.4285 | 0.9605 | 0.988 | ± | 0.23 |
| 249 | glycerol | 0.503 | 1.00 | ± | 0.0453 | 0.5154 | 0.7039 | 1.024 | ± | 0.04 |
| 251 | phosphoric 3 | 1.767 | 1.00 | ± | 0.2751 | 1.9041 | 0.811 | 1.078 | ± | 0.12 |
| 257 | valine 2 | 1.911 | 1.00 | ± | 0.0971 | 2.0916 | 0.7796 | 1.094 | ± | 0.309 |
| 259 | alanine 2 | 3.797 | 1.00 | ± | 0.1242 | 9.417 | 0.0047 | **2.48** | ± | **0.18** |

→ myo inositol +

→ glucose/fructose +

→ citric acid +

→ malic/succinic acid -
fumaric/aspartic acid (-)

→ alanine/glycine +

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