

Synthesis of metal nanoparticles in plants for the production of high-value catalysts

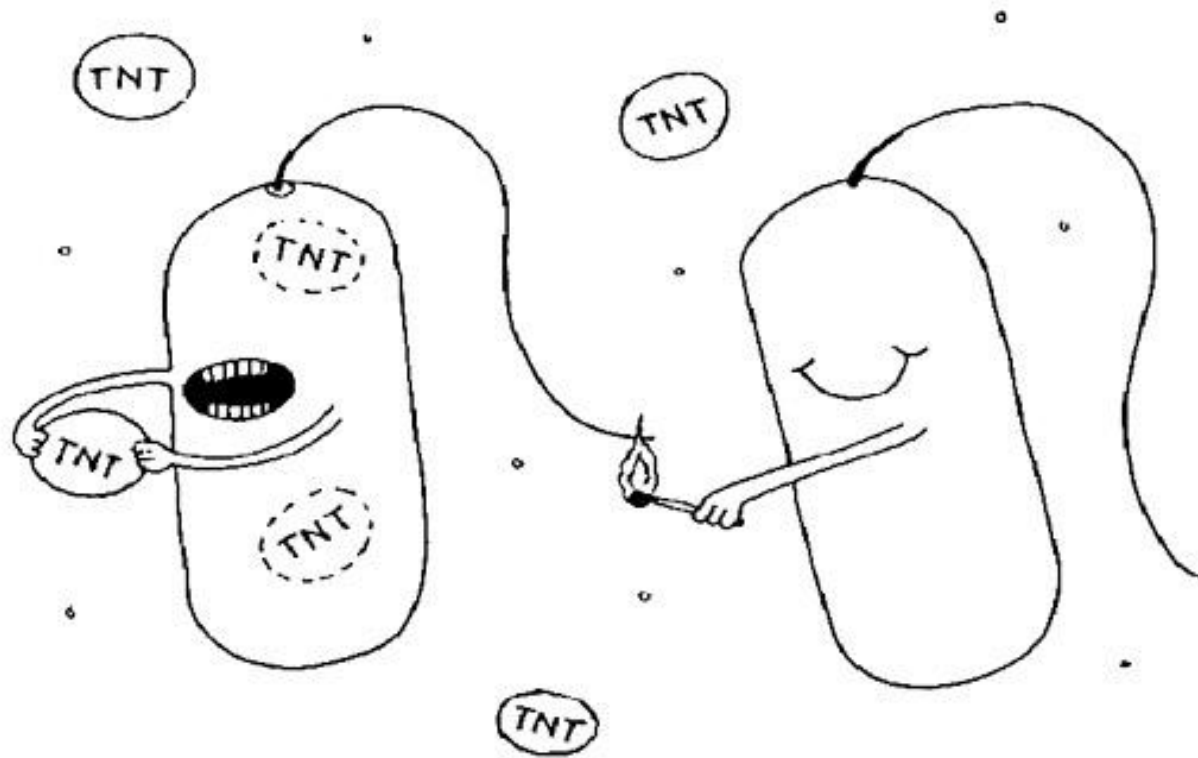
Neil Bruce

CNAP

Centre for Novel Agricultural Products

**Department of Biology
University of York
UK**

How I became interested in phytotechnologies...



PROKARYOTE PRACTICAL JOKES - THE LOWEST
FORM OF HUMOUR.

- Johnston *et al.* 2015. *Science* 349: 1072
Jackson *et al.* 2007. *Proc. Nat. Acad. Sci. USA* 104: 16822
Rylott *et al.* 2006. *Nature Biotechnology* 24: 216
Hannink *et al.* 2001. *Nature Biotechnology* 19: 116
French *et al.* 1999. *Nature Biotechnology* 17: 4918

Environmental contamination by explosives

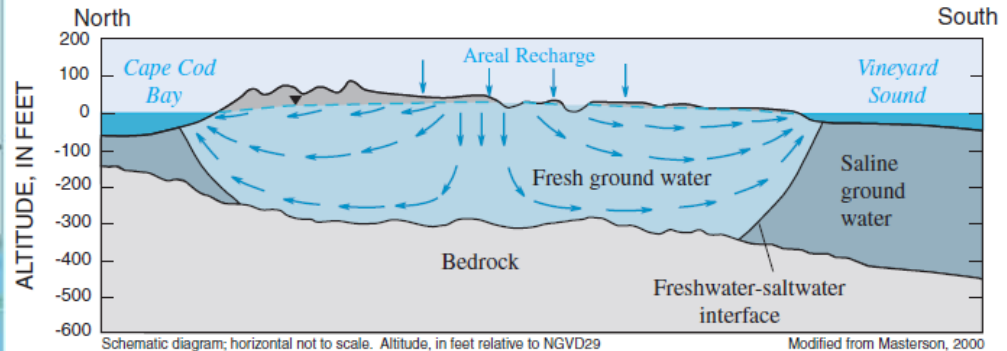
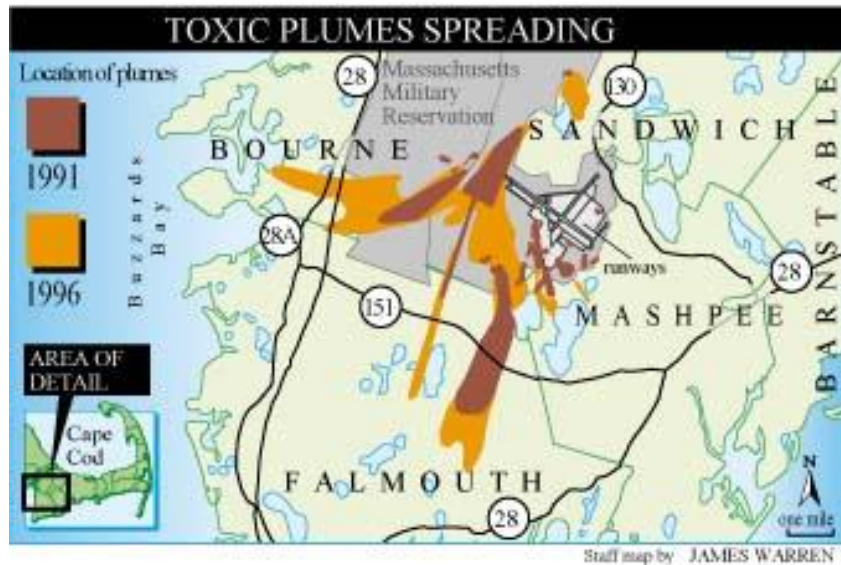




Imperial War Museum – commons.wikimedia.com

- Large scale contamination of high explosives
- Over 10 million hectares contaminated by munition constituents on active ranges
- 10 billion gallons of groundwater
- Treatment costs estimated at \$16-165 billion
- TNT is the most widespread, toxic and recalcitrant of all the explosives

Massachusetts Military Reservation



- Approximately 19 plumes detected
- High levels of RDX in groundwater as high as 370 p.p.b.
- EPA's "lifetime health advisory" for RDX is 2 parts per billion!
- Sole source aquifer
- EPA has indefinitely suspended artillery and mortar training at MMR

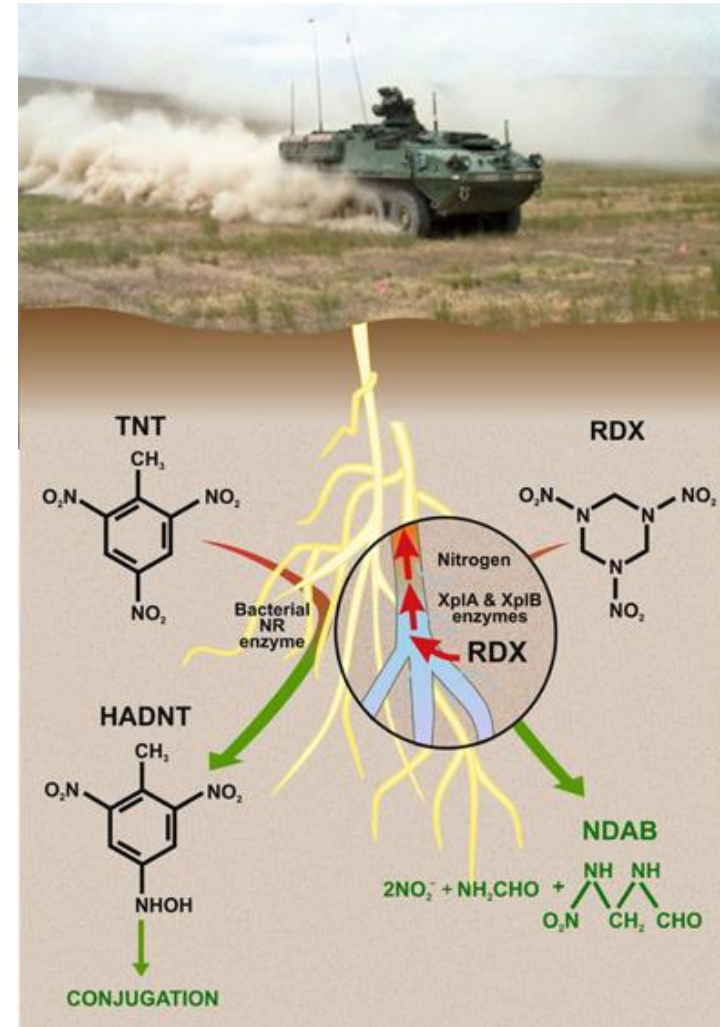
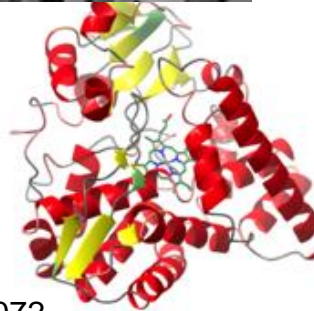
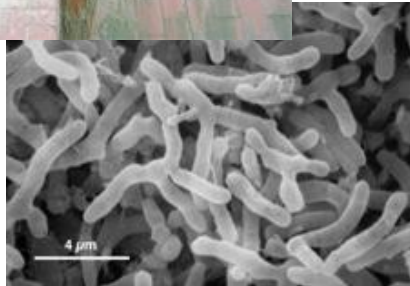
Old explosives manufacturing site



A nervous scientist!



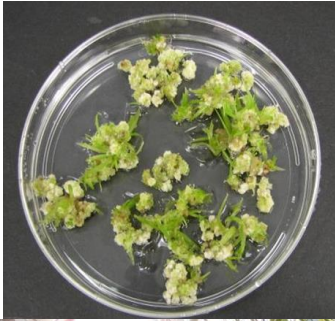
Engineering transgenic plants for the phytoremediation of explosives



- Johnston *et al.* 2015. *Science* 349: 1072
 Jackson *et al.* 2007. *Proc. Nat. Acad. Sci. USA* 104: 16822
 Rylott *et al.* 2006. *Nature Biotechnology* 24: 216
 Hannink *et al.* 2001. *Nature Biotechnology* 19: 116
 French *et al.* 1999. *Nature Biotechnology* 17: 4918

Development of transgenic grass lines

- Mature plants produced
- Switchgrass lines established in glasshouse for trials



Phytomining gold.....



Seeded with initial collaboration with Chris Anderson, Massey

scientific correspondence

Harvesting a crop of gold in plants

The possibility of turning base metals into gold has intrigued many scientists since the early alchemists, and the discovery of significant gold uptake by plants has long been a 'philosopher's stone'. But background levels of gold in plants are usually very low, rarely exceeding 10 ng per g dry tissue (10 p.p.b.). Hyperaccumulator plants, however, have 100 times the elemental concentrations of normal vegetation, a level of 1 mg per g dry tissue (1 p.p.m.). They can be used in phytoremediation¹, the *in situ* improvement of polluted sites. Hyperaccumulation can be induced by adding a chemical amendment, such as EDTA, to a plant substrate to make soluble an otherwise insoluble target metal, such as lead². Here we have induced plants to accumulate gold from ores by treating the substrate with ammonium thiocyanate. This technique might be used as a form of biological mining (phytomining) for gold^{3,4}.

We used ammonium thiocyanate as a substrate amendment because it is commonly used for making gold soluble in mining operations. Table 1 compares the ease of gold extraction in four types of ore. Unweathered sulphide gold ore (not shown in Table 1) from Macraes mine in New Zealand had very little extractable gold, and we were unable to induce plants to remove this to any significant degree. Ore from the Waihi Mine has gold mainly in its native form, which we were able to induce plants to remove. To overcome the problem of sulphide occlusion of gold in some ores and possible non-homogeneity in others, a synthetic finely disseminated colloidal gold ore (made from gold chloride) was prepared and planted with *Brassica juncea*, a plant of high biomass and rapid growth rate. Other experiments using this and other species involved the additional use of a synthetic ore prepared from finely divided gold powder (44 µm) as well as the Tui and Waihi ores crushed to a size of 0.5 mm.

All plants were grown in 250-ml pots containing the appropriate substrate. The

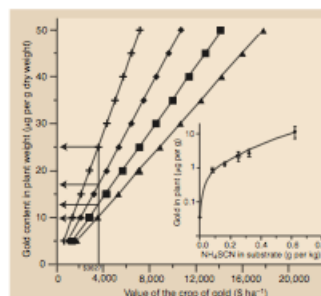


Figure 1 From gold leaves to gold leaf: the economic value of a gold crop as a function of world prices and concentrations in plant material. Prices: \$200 (crosses), \$300 (diamonds), \$400 (squares), \$500 (triangles). Inset: thiocyanate-induced uptake of gold by *Brassica juncea* from a 5 µl plant⁻¹ finely disseminated synthetic gold ore (diamonds) and natural ore (squares).

plants were treated with thiocyanate at rates of 0.00, 0.08, 0.16, 0.32 and 0.62 g per kg dry substrate weight. After seven days, aerial parts of the plants were harvested, dried and analysed for gold by graphite furnace atomic absorption spectroscopy. Gold concentrations in several plants as a function of added thiocyanate are shown in Table 1, and in Fig. 1 (inset) for *Brassica juncea* only.

All plant species showed hyperaccumulation of gold. The highest individual value was 57 µg gold per g dry weight in *B. juncea*. However, values were very variable, perhaps because the higher amendment levels caused necrosis of some plants and the induced gold concentration depended on the time that each plant remained viable. *Brassica* plants grown in the synthetic gold powder substrate (Fig. 1 inset) contained as much gold as those grown in the disseminated substrate. Similar values (9–19 µg per g dry tissue) were obtained for the same species grown in natural Waihi ore.

Induced hyperaccumulation of gold can be used for phytomining, with the resulting auriferous 'bio-ore' sold. The gross value of gold and the chemical costs involved in its extraction using a plant with a biomass of

20 t ha⁻¹ (ref. 6) are shown in Fig. 1. Assuming that thiocyanate costs \$3 per kg, the figure of \$3,627 represents its total cost per hectare when added to a depth of 15 cm at an application rate of 0.64 g per kg (the highest rate of Fig. 1). As the price of gold increases, the operation becomes more favourable economically. At the current world price of about \$300 per ounce, we would require a gold concentration of around 17 µg per g dry weight in a crop of *B. juncea*. Several values from the experiments shown in Fig. 1 were either above or very close to 17 µg per g. Some of the other phytomining costs may be recouped by selling the energy of plant combustion, as is done in the sugar-cane industry.

Induced hyperaccumulation of gold appears to be relatively independent of plant species, so it should be possible to use plants (such as chicory) that might be easy to grow on mine tailings. Any residual thiocyanate will be broken down rapidly in the substrate⁵.

We believe this is the first evidence of significant gold uptake by any plant. As well as the economic ramifications this technology may have, this ability to make a 'crop of gold' opens up the way for the phytoextraction of other noble metals.

Christopher W. N. Anderson*, Robert R. Brooks*, Robert B. Stewart*, Robyn Simcock†

*Department of Soil Science, Institute of Natural Resources, Massey University, Palmerston North, New Zealand
e-mail: r.brooks@massey.ac.nz
†Landcare Research New Zealand Ltd, Private Bag, Palmerston North, New Zealand

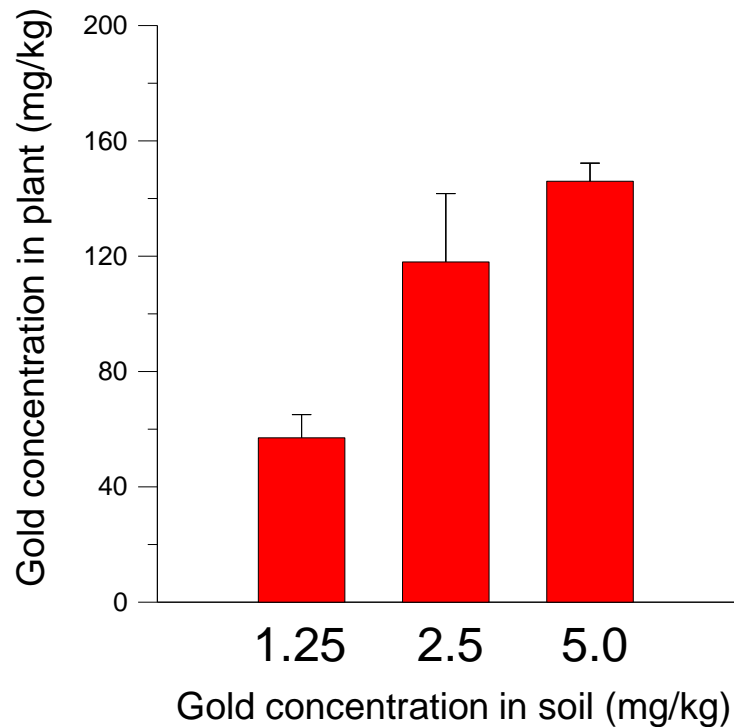
1. Dixon, C. E. In *Biological Systems in Mineral Exploration and Processing* (eds Brooks, R. R., Dixon, C. E. & Hall, G. E. M.), 371–426 (Elsevier, Amsterdam, 1995).

Table 1 Conditions and hyperaccumulation data

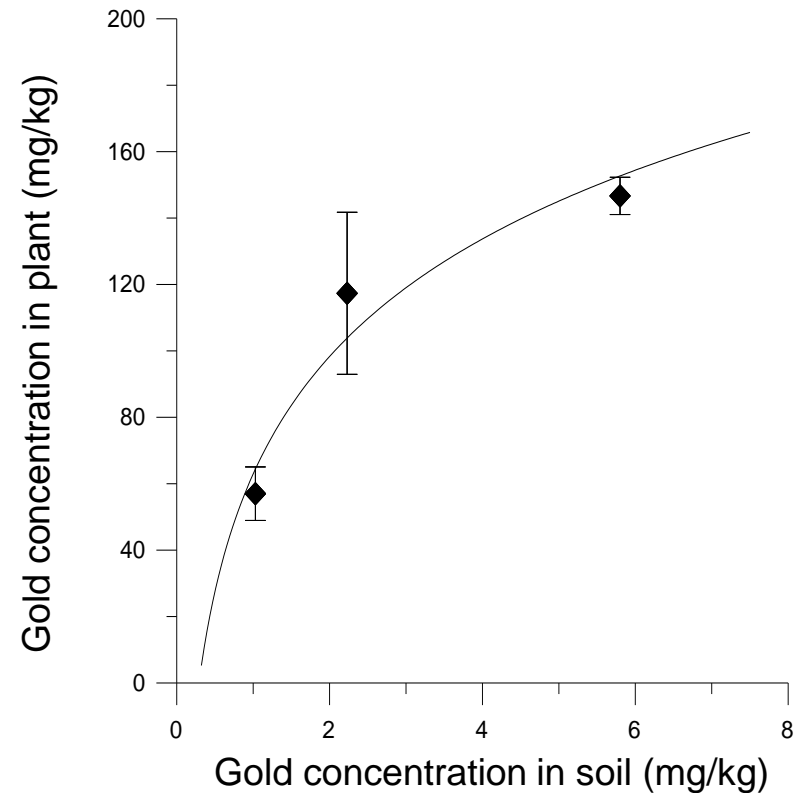
Species	Substrate	Extraction* (%)	Thiocyanate (g per kg)	Plants	Gold yield (µg per g)
<i>Brassica juncea</i>	Waihi ore†	1.8	0.50	4	8.27–19.34
	Tui mine‡	22.6	0.64	6	0.07–1.19
Chicory	Waihi ore†	1.8	0.20	1	3.09
<i>Impatiens</i> sp.	Waihi ore†	1.8	0.50	4	0.07–1.43
<i>Amorpha fruticosa</i>	Disseminated gold in sand	9.2	0.64	12	2.13–5.32
<i>B. juncea</i>	Fine gold powder in sand	–	0.25	8	0.37–6.48

*Percentage of gold extracted from 1 g of substrate into 10 ml of 2 g l⁻¹ ammonium thiocyanate in 24 h. †Gold values are for dry matter and are whole-plant analyses except for *B. juncea* on fine gold powder (44 µm) in sand, which are for leaves only. ‡Natural colloidal gold. §Acid sulphide mine tailings.

Gold accumulation in plants



Gold uptake by *Brassica juncea*
Anderson *et al.* 2003, www.gold.org



Anderson, Moreno and Meech, 2004,
Minerals Engineering



Cyanide heap-leach operation in Brazil at the CVRD Fazenda Brasileiro gold mine (now owned by Yamana Inc.)

Induced gold phytoextraction uses the same hydrometallurgical knowledge

Field trial was conducted on site



Courtesy of Chris Anderson, Massey University



Courtesy of Chris Anderson, Massey University

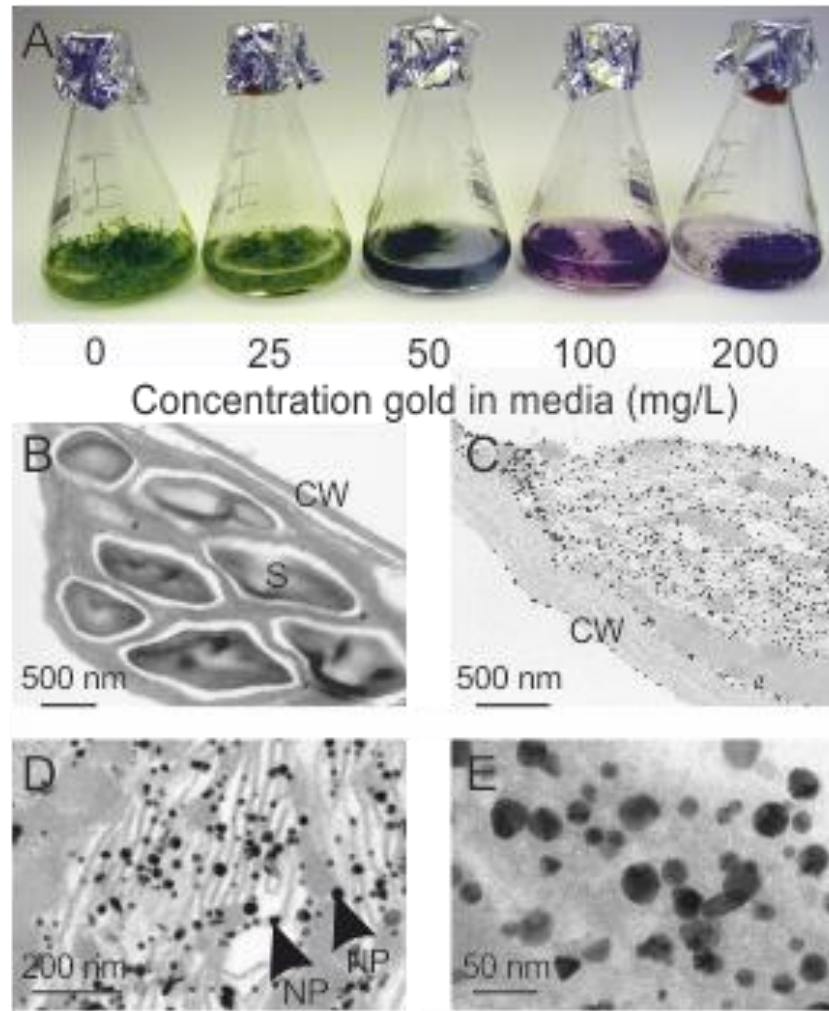


Courtesy of Chris Anderson, Massey University

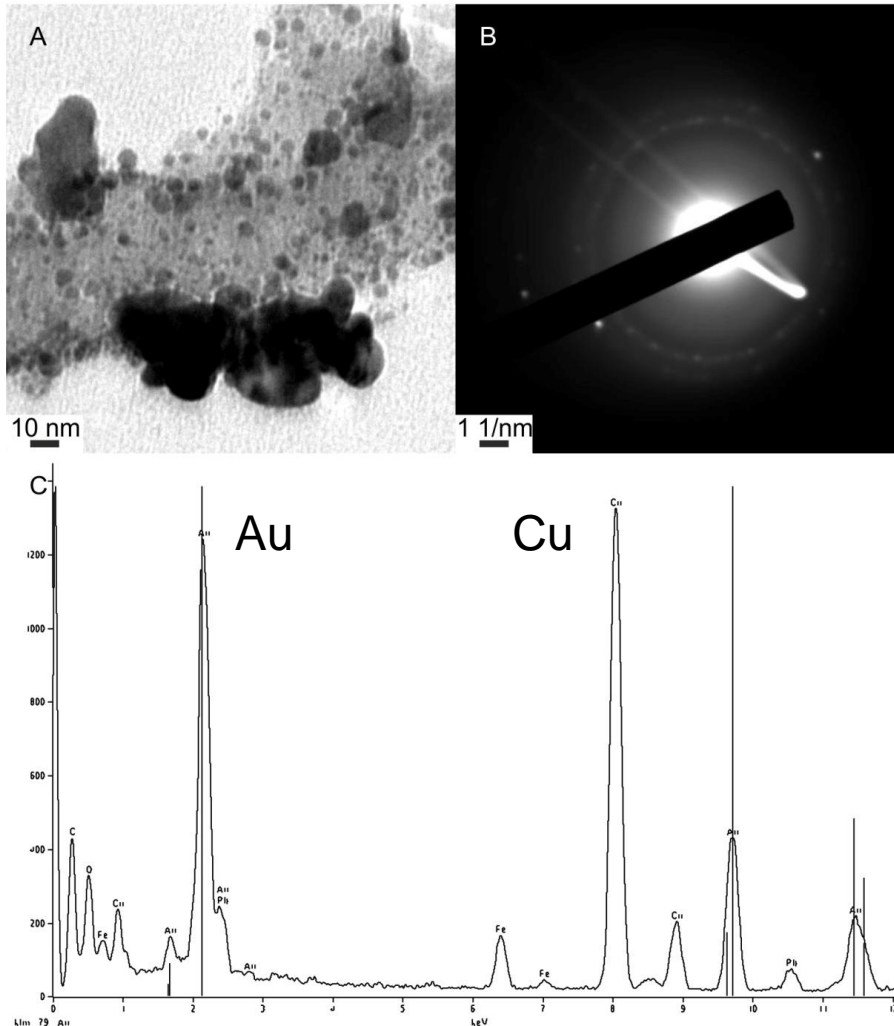


Courtesy of Chris Anderson, Massey University

Liquid-culture-grown *Arabidopsis* plants with gold chloride

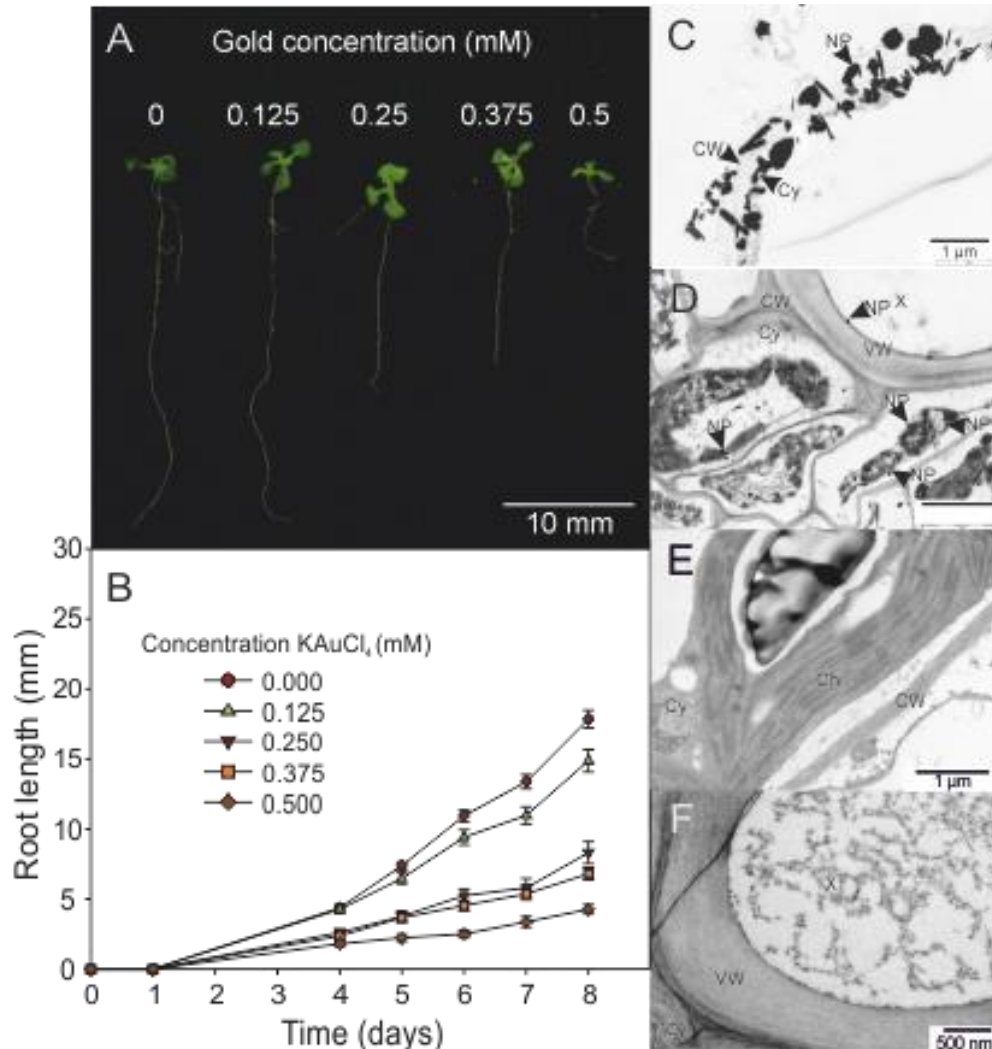


Microanalysis of gold particles



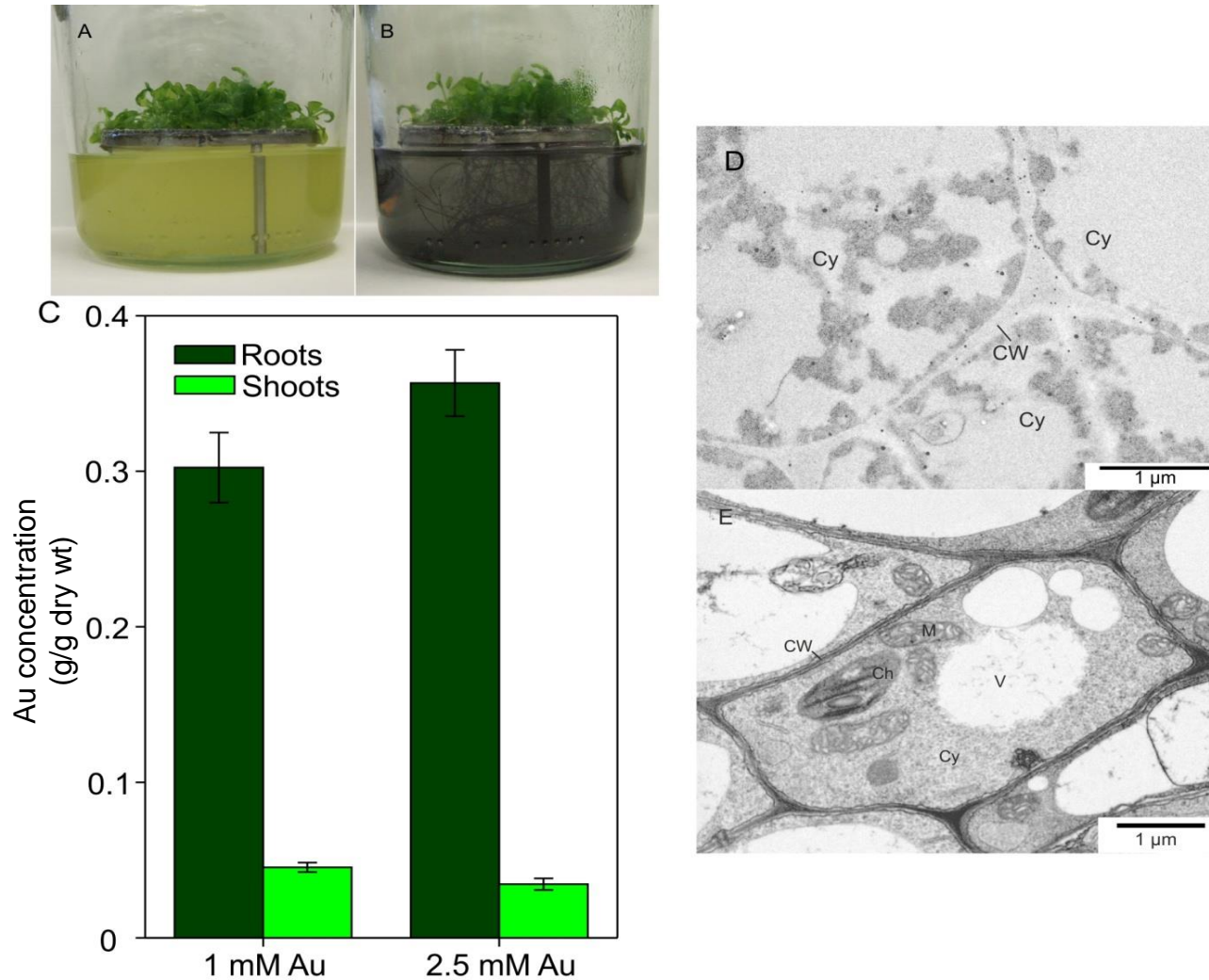
- A) Nanoparticle cluster from the roots of plants treated with 1 mM gold.
- B) Electron diffraction rings.
- C) Energy-dispersive X-ray spectroscopy (EDX) trace of the sample.

Growth of Arabidopsis seedlings in the presence of gold.

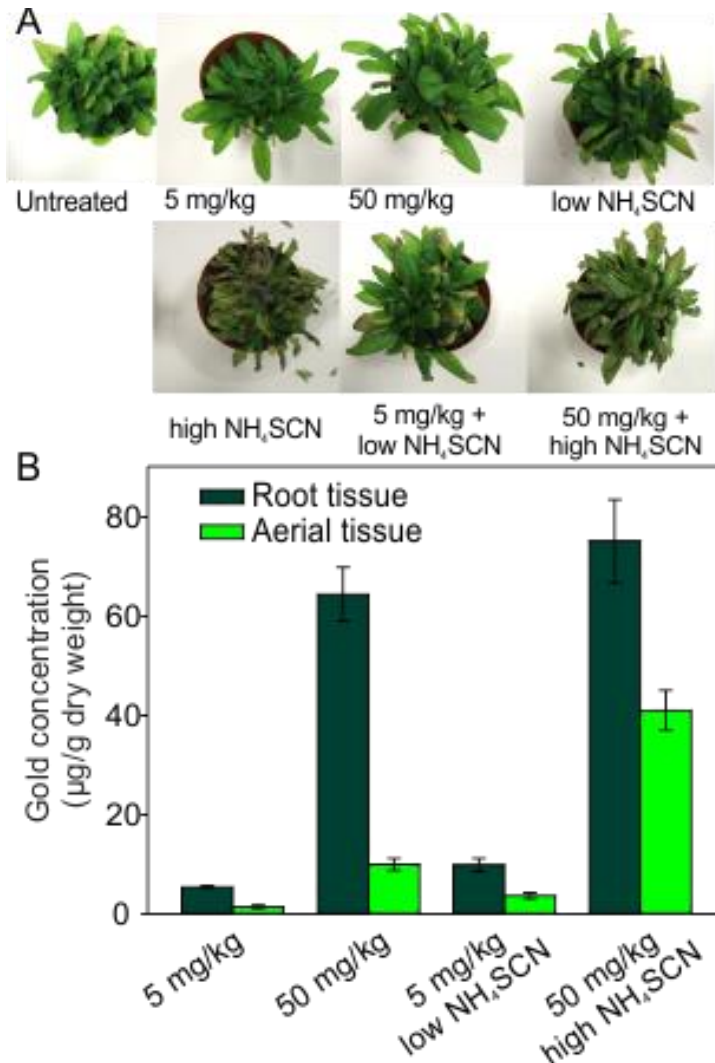


- 10-day-old seedlings grown on a range of gold concentrations.
- Scanning Transmission Electron Micrographs showing gold nanoparticles in the root (C) mesophyll and (D) vascular tissue; leaf (D) mesophyll and (E) vascular tissue.

Gold nanoparticles restricted to roots when *Arabidopsis* grown on sieves

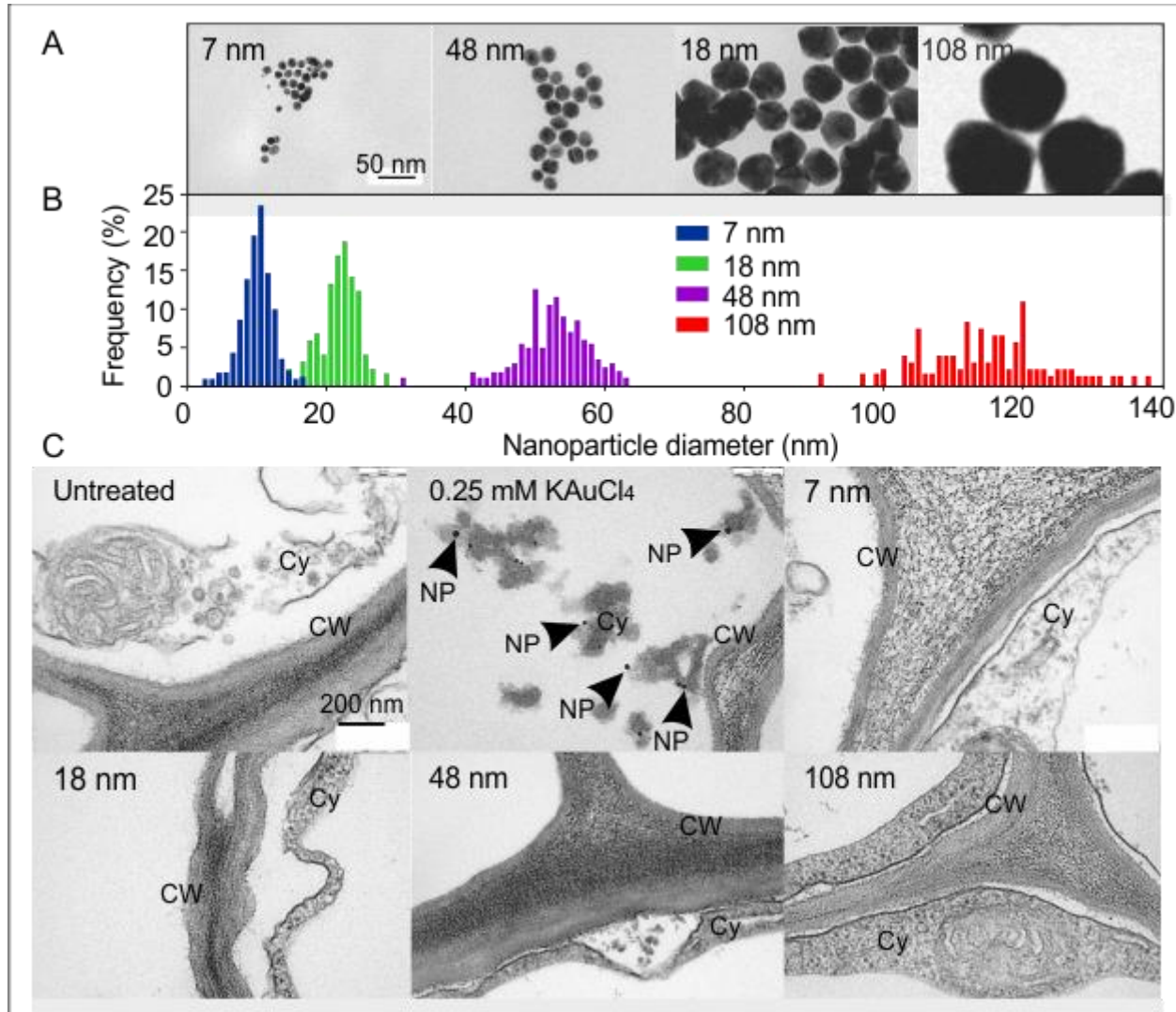


Gold nanoparticles also restricted to roots when *Arabidopsis* grown in soil



- Eight-week-old *Arabidopsis* plants grown in soil supplemented with 5 or 50 mg/kg gold (as KAuCl_4) or with no supplement. Plants were treated with water, 0.5 g/kg or 5 g/kg ammonium thiocyanate.
- Gold concentration in the root and aerial tissues from eight week old *Arabidopsis* plants grown in the presence of gold (5 or 50 mg/kg) and subsequently treated with ammonium thiocyanate or water (control).

Gold uptake- ionic or nanoparticle?



Data suggest uptake particle size < 7 nm

Elemental sustainability

PLATINUM/RHODIUM

Remaining supply:

300 years

If usage increases:

15-20 years remaining

Uses:

Jewellery, catalysts, fuel cells, catalytic converters, nuclear reactor

GERMANIUM

Remaining supply:

5 years

Uses:

Fibre-optics, infrared optics, solar cells, semiconductors

ANTIMONY

Remaining supply:

30 years

Uses:

Lead-acid batteries, semiconductor, pharmaceuticals, nuclear reactors

INDIUM

Remaining supply:

13 years

Uses:

Solar cells, LCD's, touch screens, nuclear medicine, semiconductors

HAFNIUM

Remaining supply:

20 years

Uses:

alloys, microprocessors, nuclear control rods

NEODYMIUM

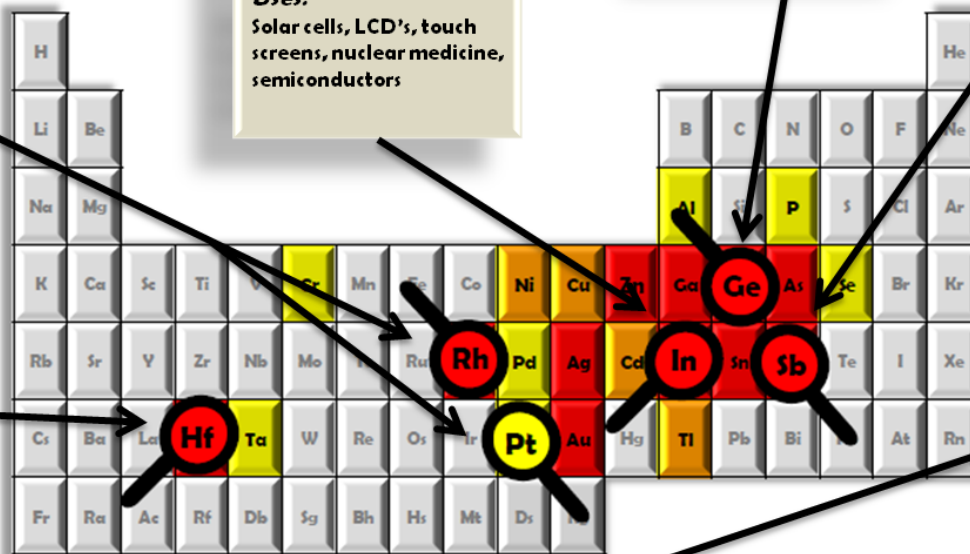
Remaining supply:

Unknown (will depend greatly on increasing usage)

Uses:

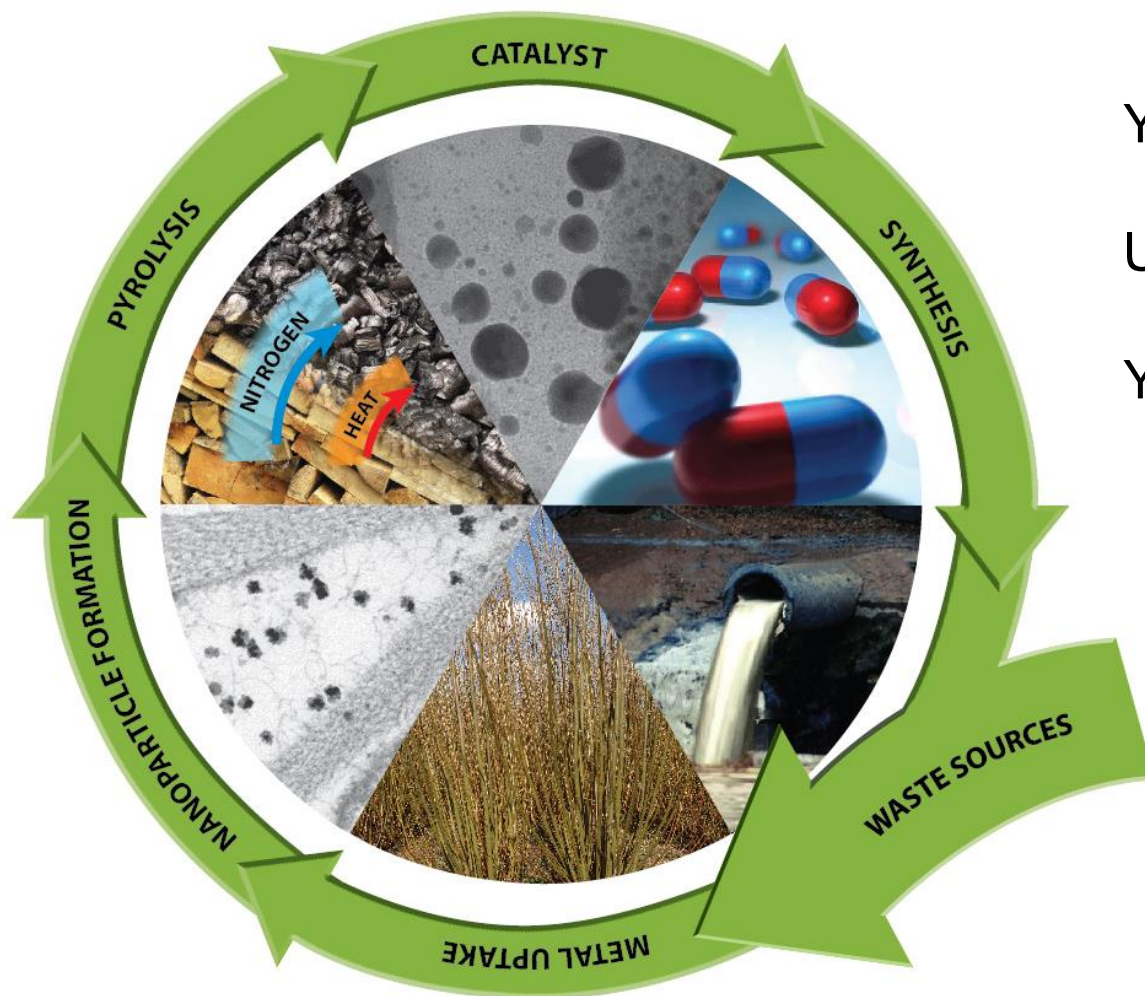
Electric motors in hybrid and electric vehicles, generators and wind turbines

NUMBER OF YEARS LEFT
IF THE WORLD CONTINUES TO
CONSUME AT CURRENT RATE



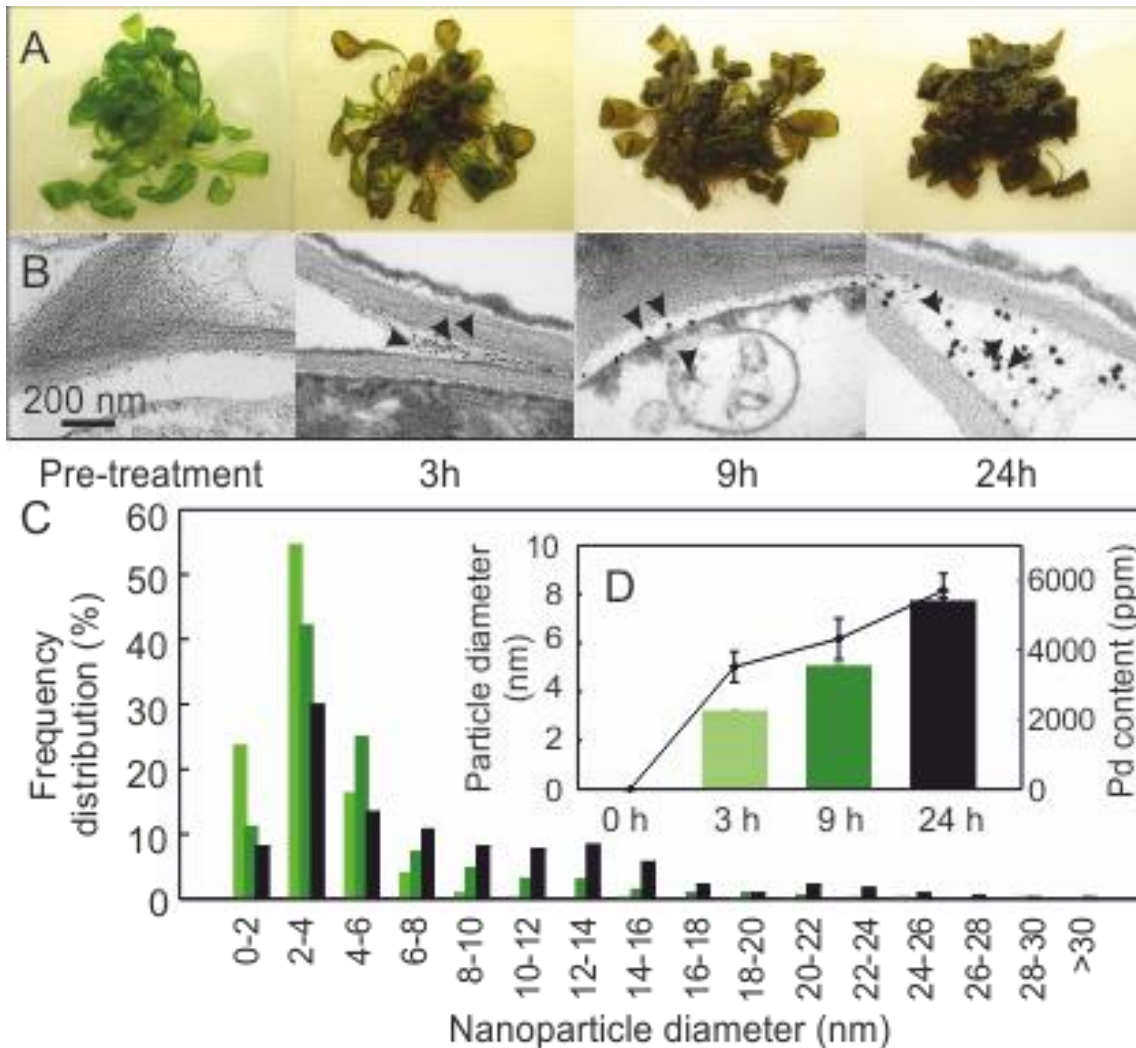


*Funded by G8 Research Councils Initiative on
Multilateral Research Funding: Material Efficiency*

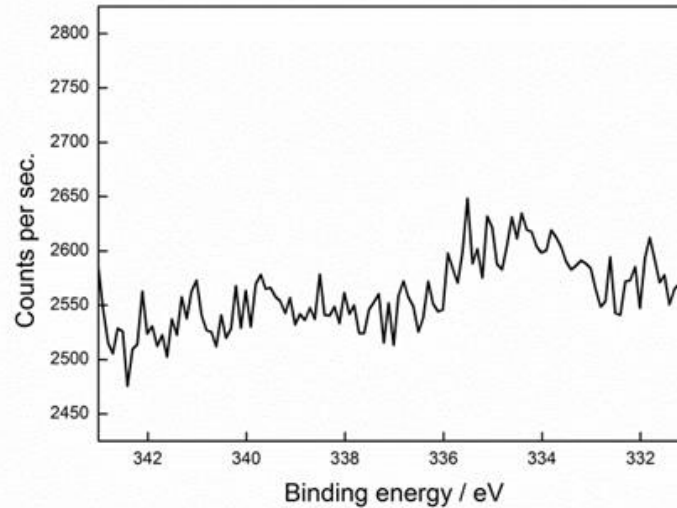


York : James Clark
Neil Bruce
UBC : John Meech
Chris Anderson
Yale : Tom Graedel

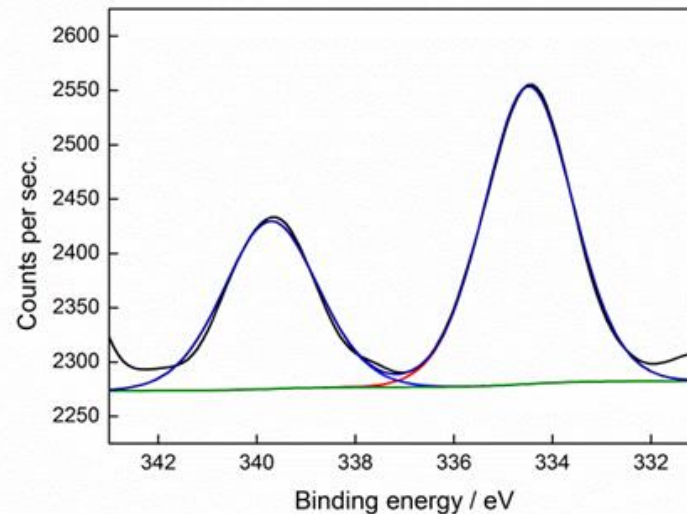
Palladium uptake and NP formation in Arabidopsis



X-ray photoelectron spectroscopy

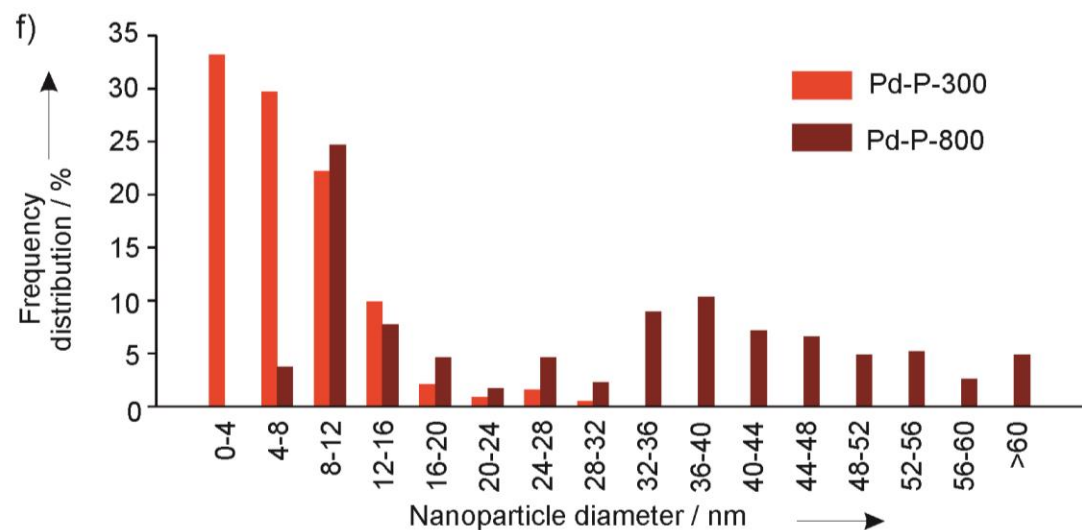
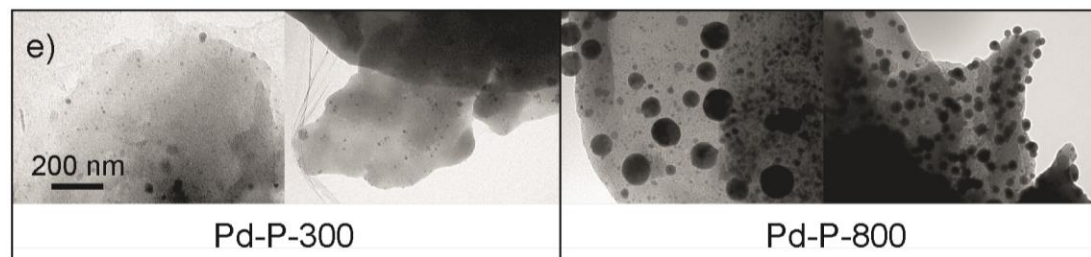


Plant without
palladium



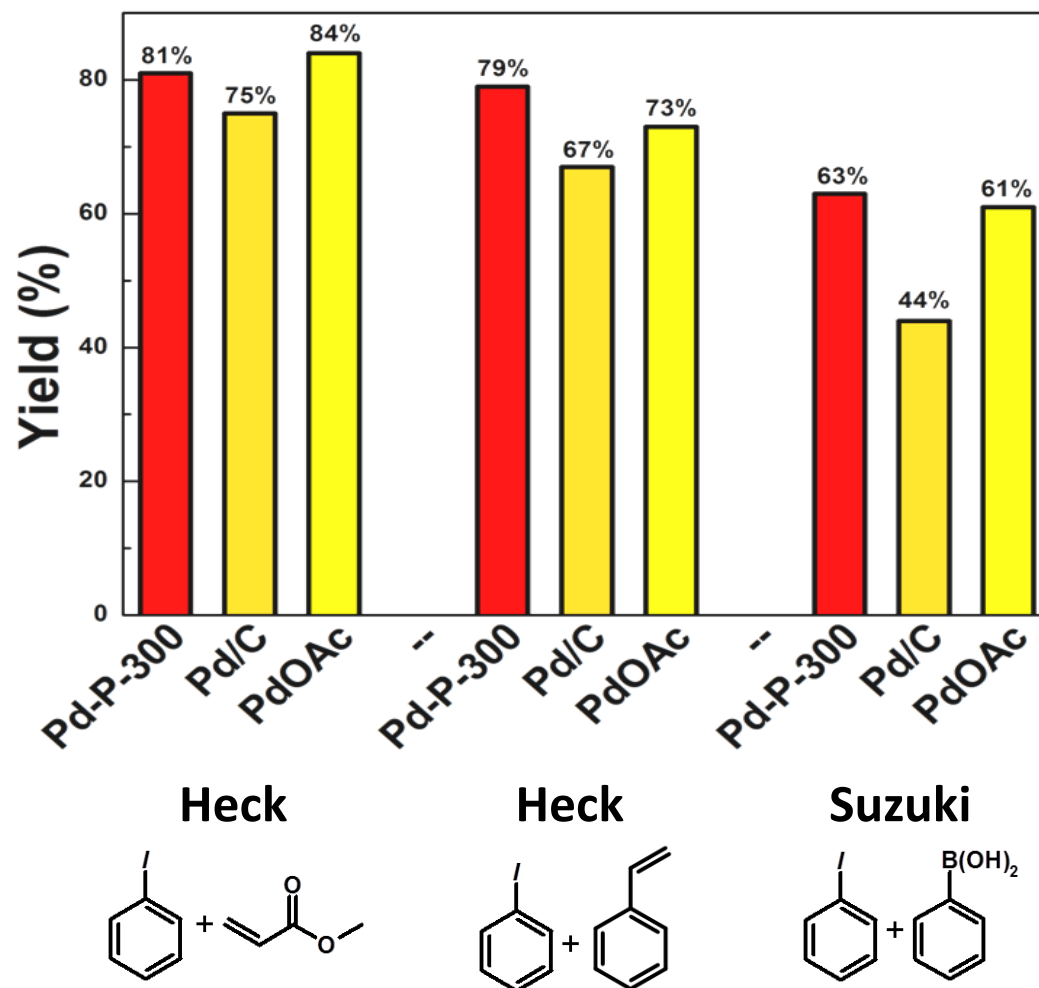
Plant containing
palladium

Distribution of PdNP sizes in the plant catalysts

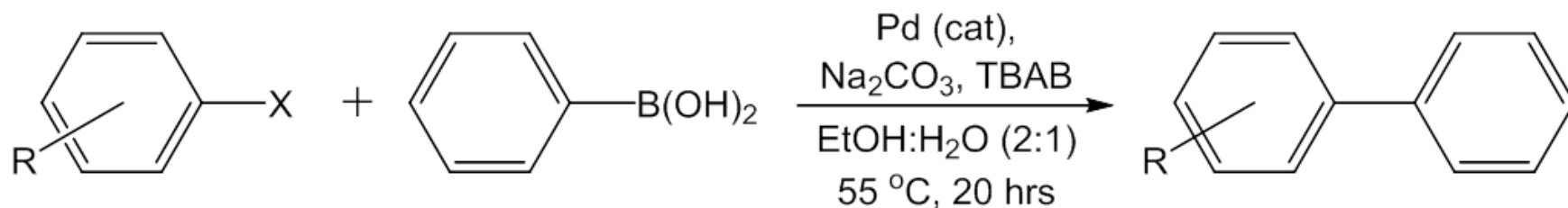


- Dried plant powder was pyrolysed under N_2 to 300 °C (Pd-P-300) and 800 °C (Pd-P-800).
- Thermal gravimetric-infrared (TGIR) analysis showed a 45% mass loss, attributed to water and carbon dioxide.
- Palladium concentration was 15% as determined by inductively coupled plasma (ICP) analysis.

Catalyst Activity: C-C bond forming reactions



Suzuki-Miyaura C-C coupling reaction of arylhalide with phenylboronic acid.



Entry	Aryl halide	Yield ^b (%)
1		100
2		98
3		99
4		93
5		98
6		94

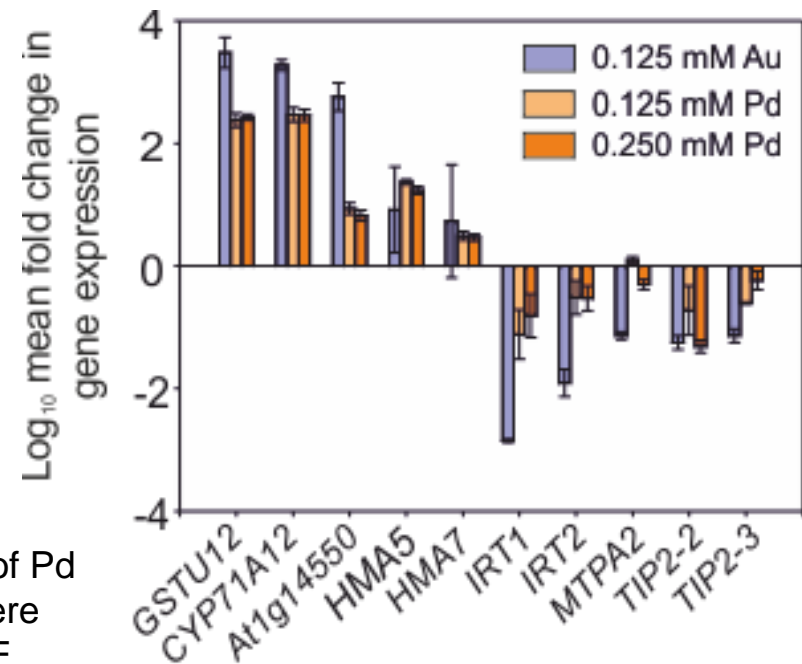
Entry	Aryl halide	Yield ^b (%)
7		79
8		100
9		99
10		99
11		99
12		81

Genetics- Arabidopsis metal transporters

Gene expression studies in Arabidopsis show that Pd treatment is triggering expression of the same suite of genes that we observed with Au treatment.



Hydroponically-grown plants were treated with Au or Pd for six hours. qPCR results, normalised to actin, were performed on root tissues. Results are mean \pm SE



Particular interest HMAs and COPT transporter genes
(Heavy metal ATPases and Copper transporters)

Arabidopsis Heavy Metal ATPases (HMAs)

Arabidopsis, heavy metal ATPases (HMAs) translocate ions across cell or organelle membranes implicated in transport of copper and silver

HMA1 - transports copper to the chloroplast thylakoids and envelope

HMA2 - transports Zn, predicted membrane transporter

HMA3 - not yet studied, predicted membrane transporter

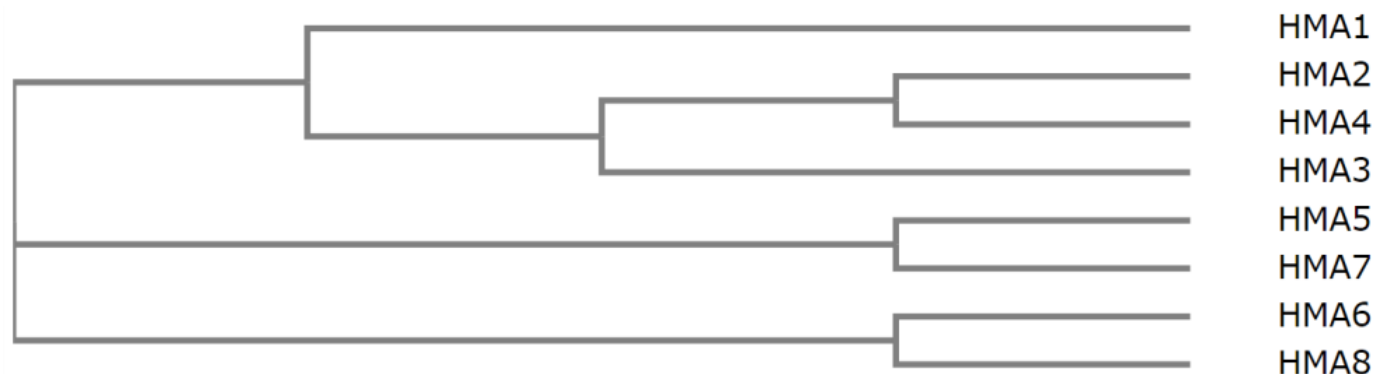
HMA4 - transports Zn

HMA5 - interacts with metallochaperones and is involved in copper detoxification in roots
(a *hma5* mutant is hypersensitive to copper)

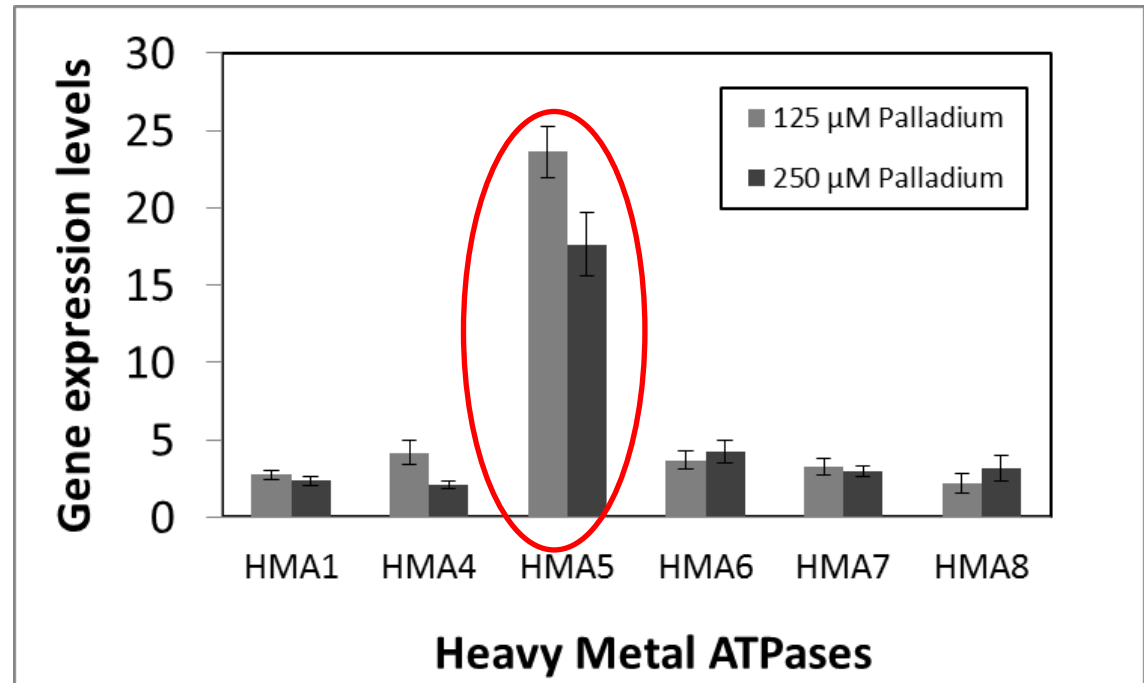
HMA6 - transports copper and, to a lesser extent, silver in the inner chloroplast envelope

HMA7 - transports Cu

HMA8 - copper transport to the chloroplast thylakoids and envelope

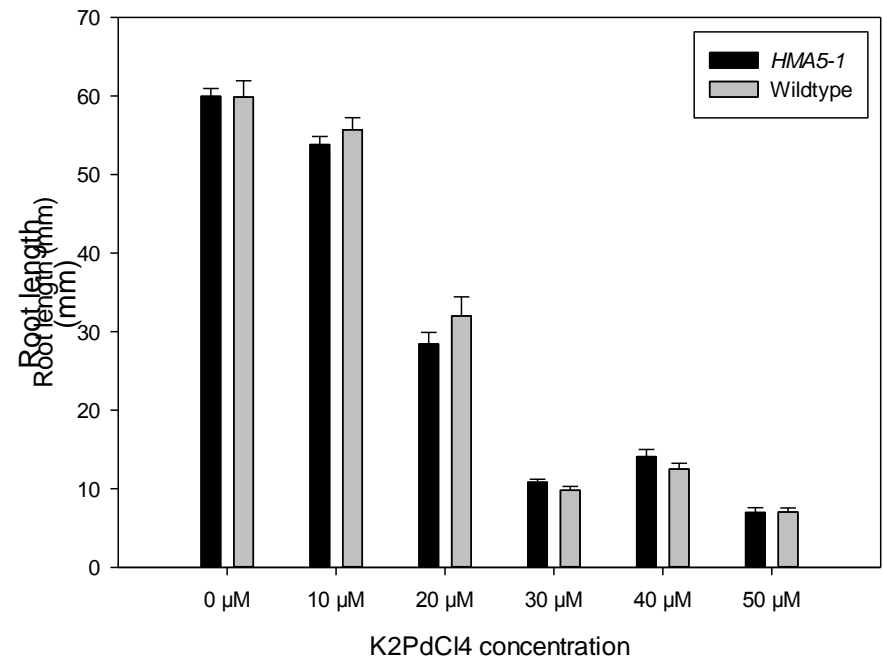
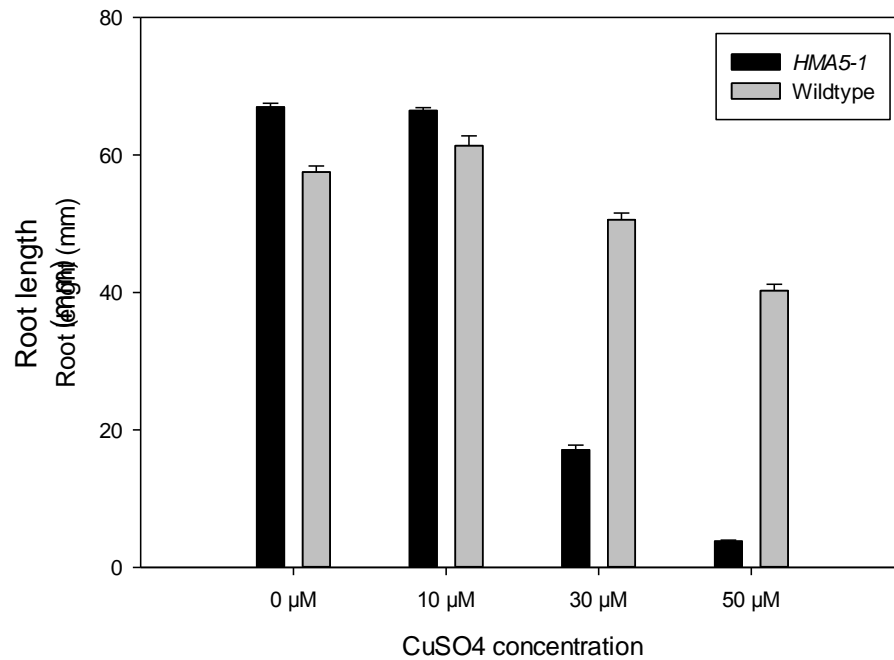


Gene expression studies on the HMA gene family in *Arabidopsis* roots fed palladium

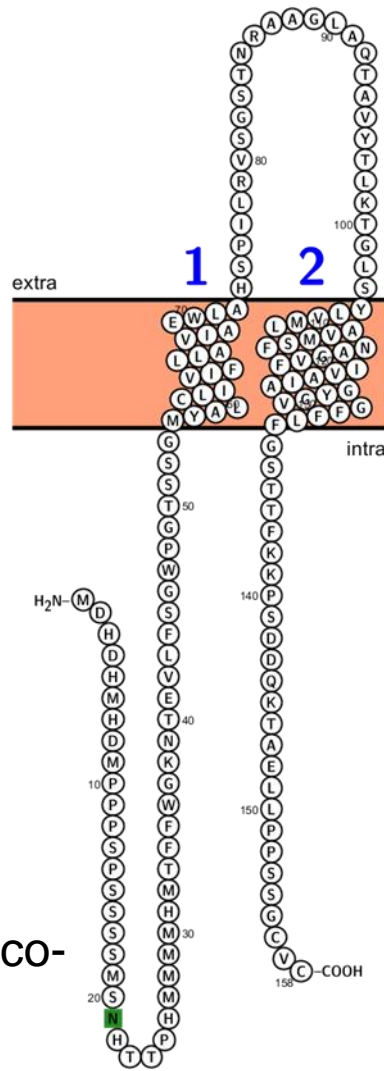


- HMA5 is most upregulated of the family (significantly higher than HMA1,2,6,7 and 8)
- HMA 2 and HMA 3 not yet tested
- Palladium in form of K_2PdCl_4

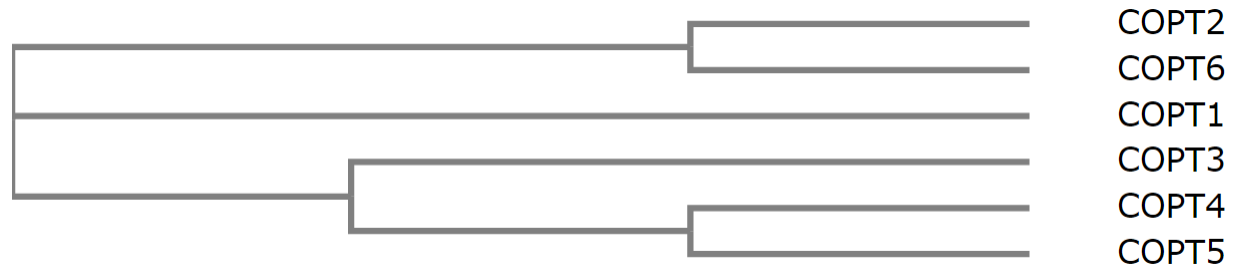
Characterisation of *hma5-1* mutant knock out lines



COPT transporter family also of interest



- COPT2 Gene expression responds to gold (23-fold upregulated)
- Small gene family (six members)
- Small protein size
- Not well studied; COPT1 uptake and distribution throughout the plant
- COPT3 and 5 control intracellular copper levels via vacuolar storage.



Summary

- Arabidopsis produces Au and Pd nanoparticles
- PdNPs show excellent catalytic activity across a range of Suzuki-Miyaura reactions
- There is a need to test other plant systems with waste streams containing Pd
- Current plant systems require high Au and Pd concentrations

Acknowledgements



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