

The potential of transgenic plants for phytoremediation

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- A forecast made 10 years ago and published in Marmioli and McCutcheon, 2003
- (in McCutcheon and Schnoor (eds), Wiley Interscience).
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Plant types relationships with advantages/disadvantages for phytoremediation

Increased human and ecological risk



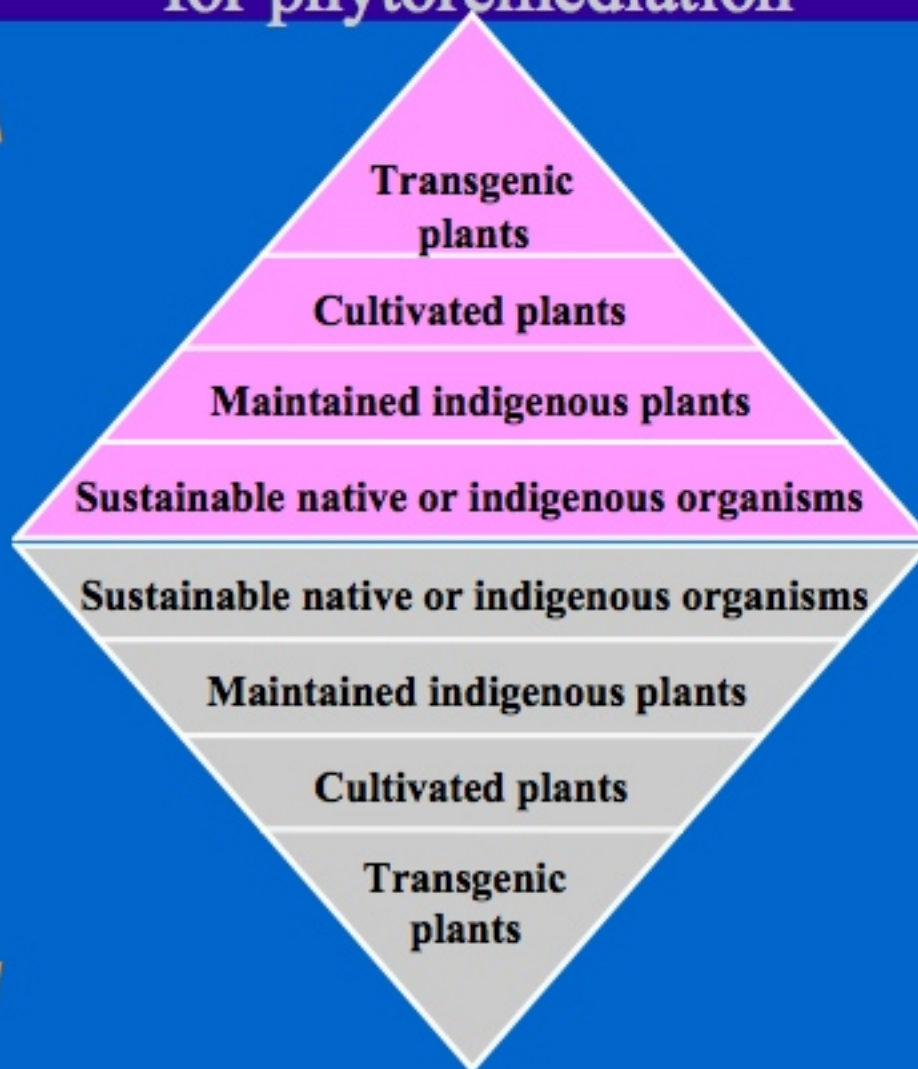
Increased genetic engineering



Increased maintenance, monitoring, and control required



Increased residual disposal



Transgenic plants

Cultivated plants

Maintained indigenous plants

Sustainable native or indigenous organisms

Sustainable native or indigenous organisms

Maintained indigenous plants

Cultivated plants

Transgenic plants

Progress in phytoremediation

- **Six years after the publication by Marmiroli and McCutcheon, it seems the progress is taking exactly the predicted pace**
- **After the years of the native/indigenous plants, it is now the time of cultivated plants**
- **These cultivated plants incorporate a certain level of genetic modification**

Use of cultivated plants

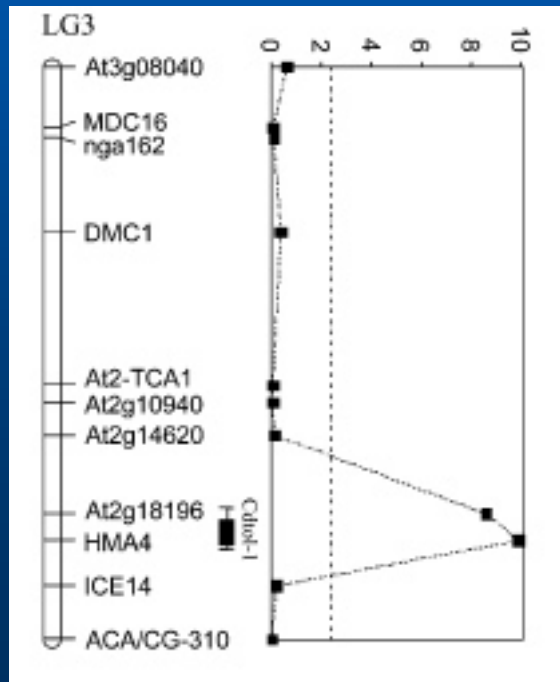
- **The use of cultivated plants in phytoremediation is having several advantages over the use of native plants, because of:**
 - Higher biomass production
 - Faster growth rate
 - Possibility for application of advanced agronomic protocols
 - Less difficulty in controlling the environmental dispersal (environmental control)
 - Recovery of byproducts (added value)
- **But also some disadvantages**
 - More attractive for animals
 - More requiring in terms of treatments (pesticides, fertilisers, watering)

The value of genetic analysis of natural plants

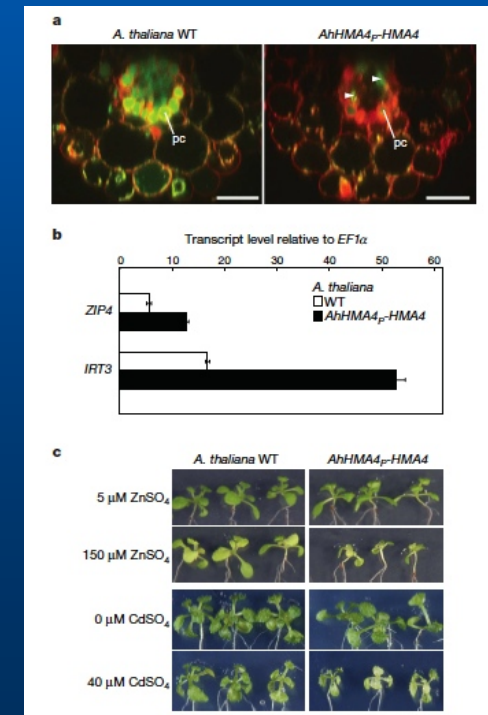
- It has provided an understanding of the functions involved in phytoremediation which otherwise would have been difficult to achieve at metabolic level only through biochemical analysis
- It has allowed the transfer of information to the use of cultivated plants
- It has identified genes for the production of genetically modified plants for phytoremediation:
Trait Specific Phyto-plant = TSP (instead of GMP)

Top down vs bottom up approach - HMA4 (plasma membrane metal pump)

- **Bottom-up (Courbot et al., 2007):** studying an interspecific cross between *A. halleri* and *lyrata*, a QTL for Cd tolerance was found. The gene HMA 4 cosegregates with the QTL.



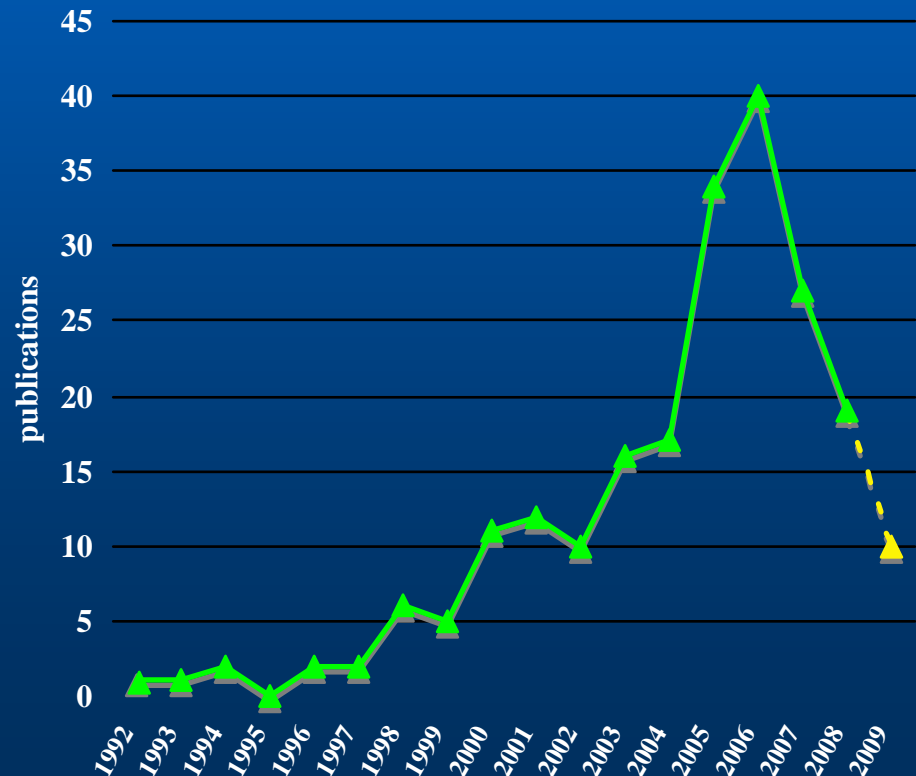
- **Top-down (Hanikenne et al., 2008):** expression of the HMA4 gene in *A. thaliana* confers a similar phenotype of tolerance to Cd and Zn as *A. halleri*.



Strategies of genetic engineering in plants

- In all cases, two main strategies have been applied for improving phytoremediation by means of genetic engineering
 - Transformation with plant genes: **enhancing existing properties**
 - Transformation with genes derived from other species (plant, animal, microorganism): **conferring new properties**

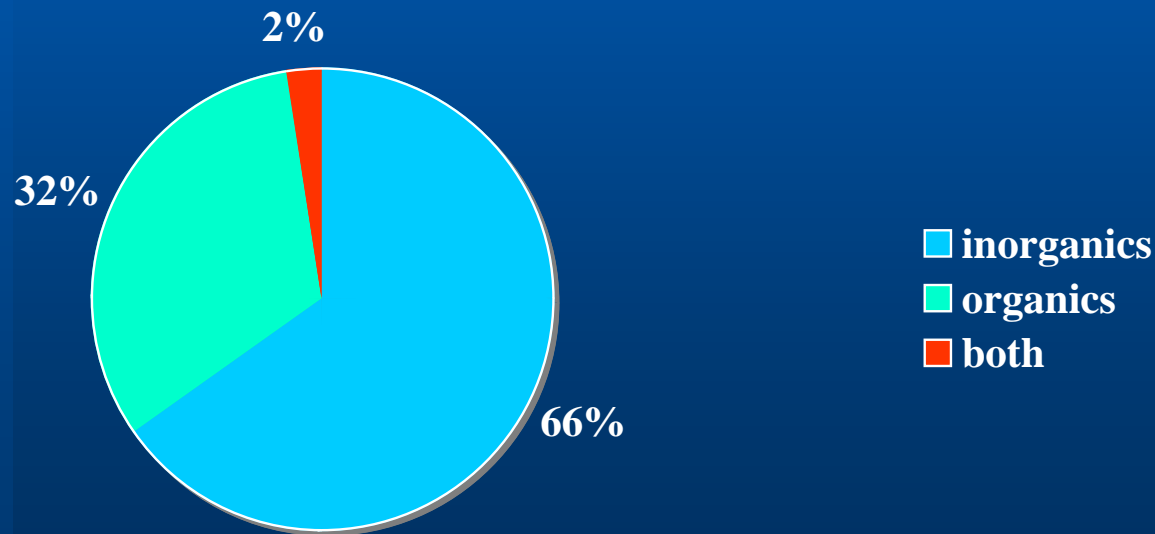
Publications along 18 years



Contaminants

Summary from 216 papers, 1992-2009

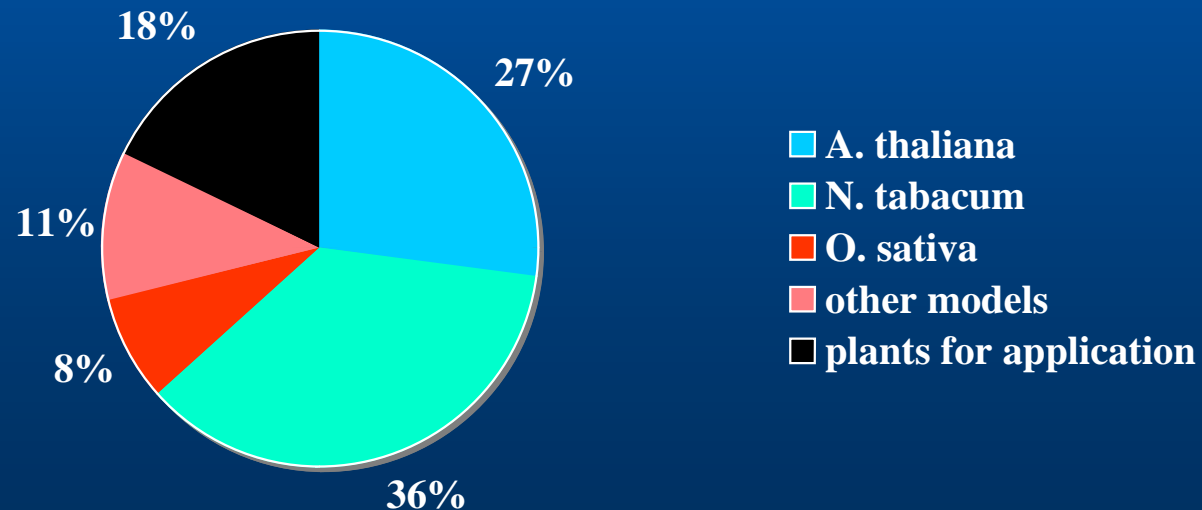
Most papers on transgenic approaches have addressed inorganics, such as Ni, Cd, Hg, As, Zn. Organics tested were mainly pesticides, herbicides, explosives, organomercurial compounds. Few papers addressed responses to both classes of contaminants at the same time.



Recipient plants

Summary from 216 papers, 1992-2009

The plants used for experiments were mainly tobacco and *Arabidopsis*. Rice was instead used for most experiments with pesticides. Other model plants included potato and tomato. Plants of applied relevance were mainly poplar and *Brassica juncea*.



Main targets for genetic engineering - inorganic contaminants

- **Transporters**
- **Metabolism of chelators and sulfur metabolism**
- **Storage in sinks**
- **Se-cysteine methyltransferase and Se-Cys lyase, and Cystathionine-gamma-synthase**
- **Enzymes for ionic mercury reduction from bacteria**
- **Arsenate reductase from bacteria**
- **ZntA pump from bacteria**

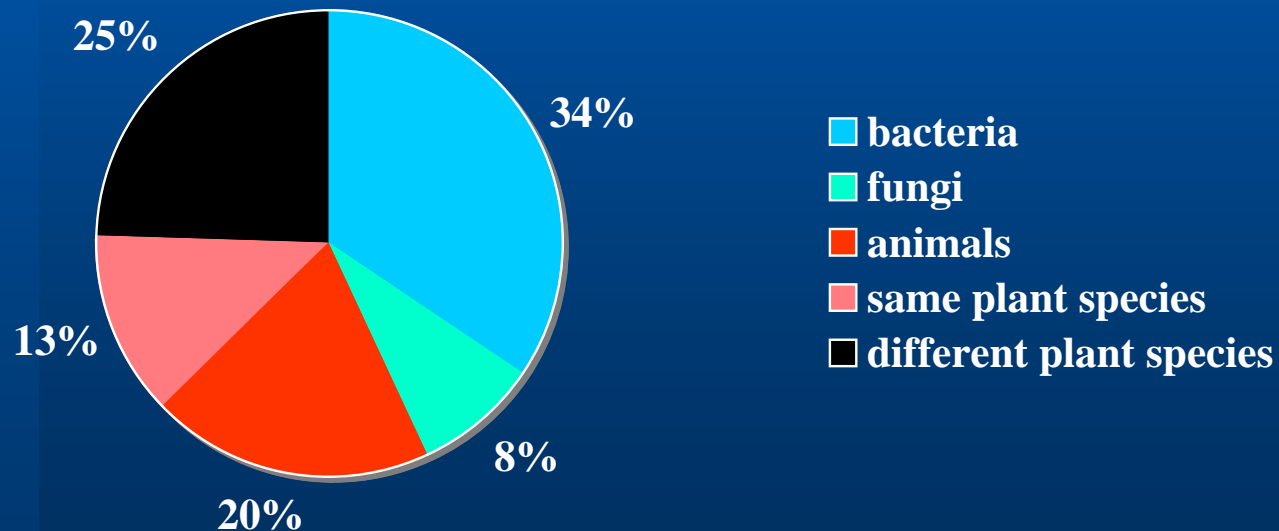
Main targets for genetic engineering - organic contaminants

- **Degradation of specific compounds**
- **Phase I of xenobiotic metabolism**
- **Phase II of xenobiotic metabolism**
- **Mn peroxidase for PCP**
- **Peroxidases for phenolics**
- **Laccase for phenolics**

Source of transgenes

Summary from 216 papers, 1992-2009

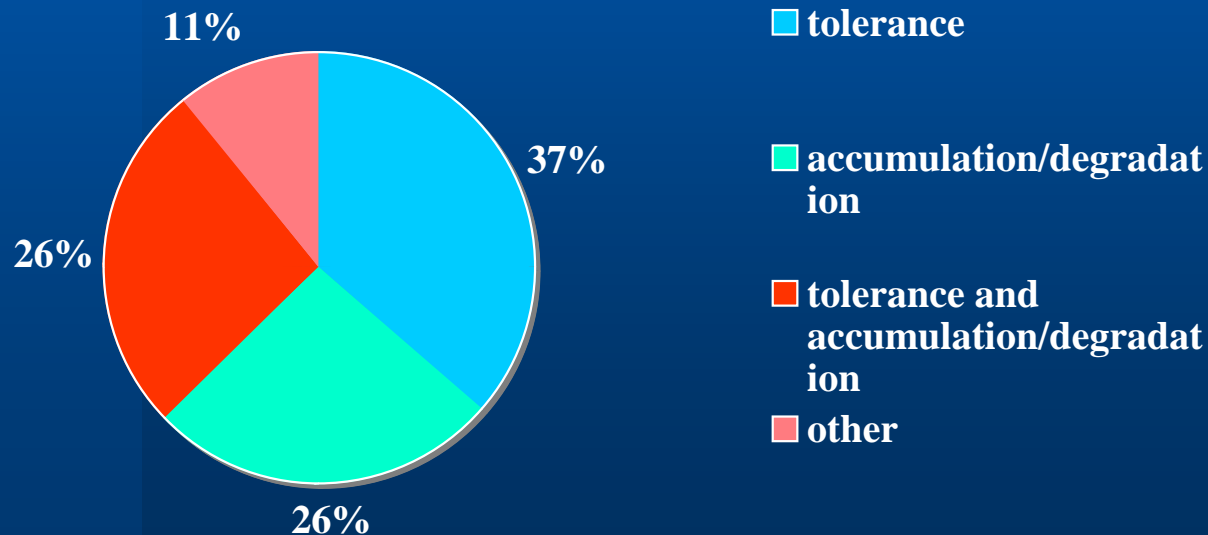
The main source for heterologous genes was found in bacteria, both for organics and inorganics phytoremediation. Animal sources include mammals and human. Plant genes were mainly introduced in *Arabidopsis* and tobacco coming from other plant species.



Effects

Summary from 216 papers, 1992-2009

The main effect obtained in transgenic approaches has been the increase of tolerance. One quarter of experiments report increase in accumulation of inorganics or degradation of organics together with increased tolerance. But many experiments lead to no changes, or even to results opposite to expectations.



Successful field experiments

PLANT	GENE	FIELD TRIALS	YEAR
tomato	esterase	Resistance to thiazopyr	1997
tobacco	Mammal metallothionein	Decreased Cd content in leaf	1998
Indian mustard	ATP sulfurylase, APS	Increased Se accumulation	2005
Indian mustard	Gamma-glutamyl-cysteine synthetase, ECS	Increased Se accumulation	2005
Indian mustard	Glutathione synthetase, GS	Increased Se accumulation	2005
rice	Cytochrome CYP2B6	Removal of herbicides from soil	2005
rice	Cytochromes CYP1A1, 2B6, 2C19	Removal of herbicides from soil	2006
Indian mustard	Selenocysteine lyase, cpSL	Increased Se accumulation	2007
Indian mustard	Selenocysteine methyltransferase, SMT	Increased Se accumulation	2007
cottonwood	Mercuric ion reductase, merA	Increased Hg resistance and volatilisation	2007
rice	Nicotianamine synthase, NAS	Tolerance to low Fe in soil	2008
rice	Cytochromes CYP1A1, 2B6, 2C19	Removal of herbicides from soil	2008
poplar	Glutathione biosynthesis, gsh1	Observed differences	2009
rice	Fe transporter, OsIRT1	Increased Fe and Zn content	2009

Patenting

- **About 30 patents protect the design of transgenic plants for phytoremediation. Target genes are:**
 - glutamylcysteine synthetase (metal)
 - sulfate assimilation pathway enzyme gene (metal)
 - arsenate reductase (metal)
 - Acyl-CoEnzyme-A-Binding Proteins (metal)
 - rhamnosyltransferase activity (organics)
 - P1B-type ATPase (metal)
 - ATP-binding cassette (ABC) transporter protein (metals, organics)

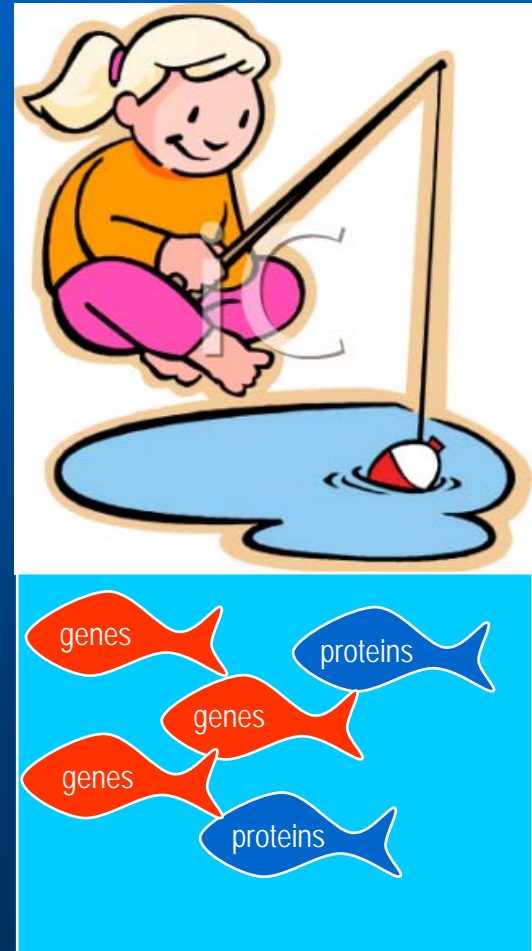
Limits of plant genetic engineering



- In classical genetic studies, plants are crossed and the resulting variability is analysed; this may be more or less complicated, but the cross always “succeeds”
 - The real problem is interpreting the segregation of traits
- In genetic engineering, instead, success depends upon the efficacy with which the isolated element is expressed in its new context
- In order to increase efficacy of expression, attention must be paid to several factors included in the so called “expression cassette”:
 - The coding sequence has to be correctly translated, and this may require modification of codons
 - The promoter sequences driving gene expression have to be efficient in the host species, possibly conferring tissue-specific expression
 - Other surrounding gene sequences in the construct have to be tailored according to the host species
- Genetic engineering in plants has a limited range of pyramidization and has not been used to transfer complex traits (QTLs, quantitative trait loci)

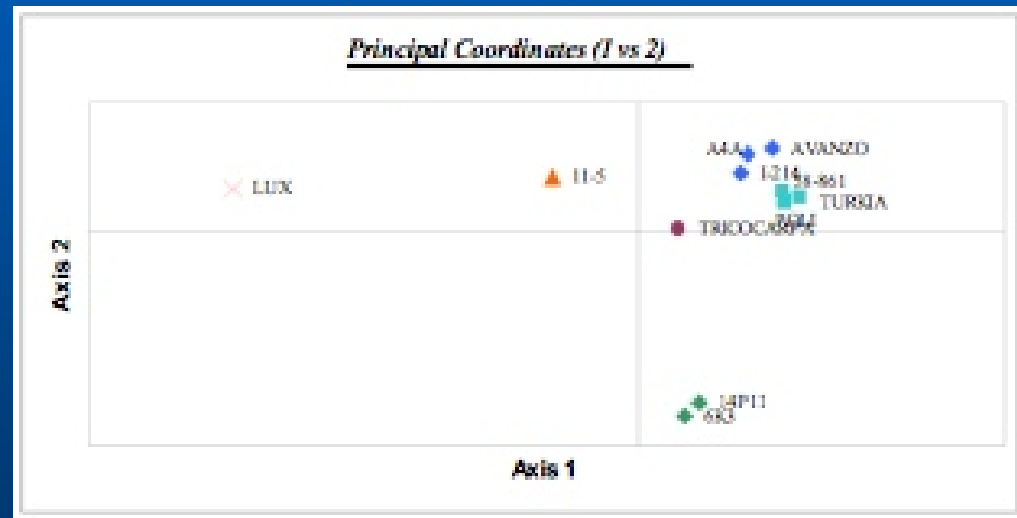
Phenotypic vs genotypic plasticity

- **Adaptation to contaminants can result either from**
 - Genotypic plasticity (gene variants)
 - Phenotypic plasticity (variation in expression of proteins and metabolites)
- **Genetic engineering is like fishing in a pond**



Fishing for genetic variation

- Poplar clones selected for differences in tolerance and accumulation to Cd show single nucleotide polymorphisms in the coding sequences of genes involved in transport and chelation of metals.
- Genetic variants could be correlated to changes in protein sequence and function, eventually.
- Genetic variation classifies the clones into clusters, correlating with tolerance/accumulation of cadmium



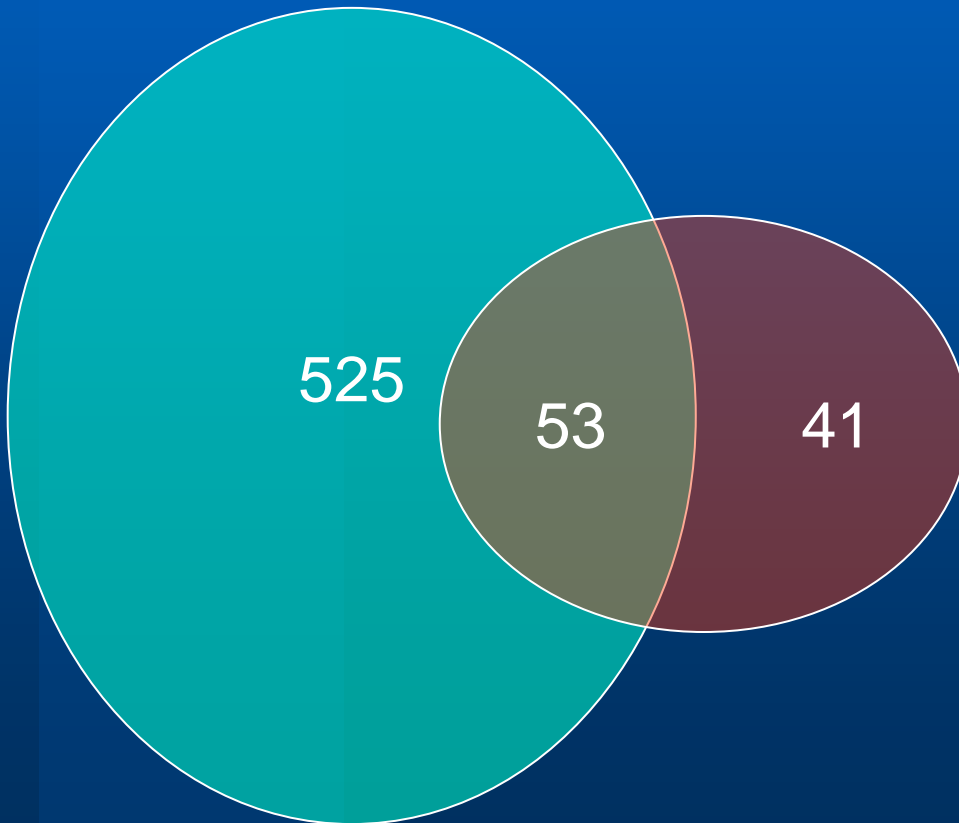
Phenotypic plasticity

- **Transcriptomic studies of gene expression identify hundreds of genes differentially expressed, but are they all important?**
- **Proteins are more directly related with phenotypes, and therefore to adaptation**

Transcriptomics vs proteomics (metal tolerance/accumulation)

TRANSCRIPT
PROFILING

PROTEOMICS



Until now transcript profiling has identified more functions as compared to proteomics

- It has a high resolution capacity, because it provides data about thousands of genes at the same time
- Proteomics leads to identification of hundreds of proteins at best
- It detects transcripts expressed at extremely low levels
- Proteomics detects only some of the proteins, based on extractability and expression level relatively high

Proteomics studies for metal response

gene name	POPLAR	THLASPI	LICHEN
chaperone clpB	induced		
HSP60			induced
DNAJ chaperone			induced
ATP dependent Clp	induced		
dehydration responsive	induced		
stress induced KIN2		repressed	
cytochrome P450	induced	induced	induced
glutathione s transferase		induced	induced
aquaporin	induced		
ZRT/IRT like		induced	
metallothionein 1B		induced	
thylakoidal processing peptidase	induced		
rubisco large subunit	repressed		
chlororespiratory reduction protein		repressed	
Myb transcription			induced
ras related rab7		induced	
protein kinase APK1B	induced		
auxin responsive IAA2B		induced	
phloem protein A6		induced	
hydroxyacylglutathione hydrolase I	induced		
NADPH isocitrate dehydrogenase		induced	
acyl carrier protein 2		induced	
ATL4C precursor		induced	
leucine zipper 16		induced	
geranyl geranyl pyrophosphate synthetase		induced	
thioredoxin H type		induced	
aspartate aminotransferase		induced	
serine threonine dehydratase			induced
glutamate aspartate transport			induced

Stress response

Xenobiotic metabolism

Metal related

Chloroplast

Gene regulation

Other metabolic,
transport, etc.

Conclusions

- When thinking about genetic engineering plants for phytoremediation, a functional approach can be considered to solve the problems (e.g. QTLs) and the inconsistencies (e.g. limited genetic variability)
- Proteomic can give specific targets, whose role is undoubtedly established, in time
- Therefore proteins can be used in refining the genetic engineering approach by:
 - Limiting the number of targets
 - Defining precisely the targets themselves

Not only. Other facts have to be considered.

THE “DESIGN SPACE”

Economic factors	Geology, hydrology
Ecological aspects	Biological criteria (genetic, biochemical, physiological)

The “design space” is the holistic transducer of our knowledges. The matrix that allows different sources of knowledges be brought together, with each discipline bringing its own contribute.



THANKS FOR ATTENTION