Cadmium (Cd) is a widespread heavy metal, released into the environment by heating systems, metal-working industries, waste incinerators, urban traffic, cement factories and as a contaminant of phosphate fertilizers. Cd is one of the four metals that have been mentioned to be a world-wide concern in terms of their importance in environmental quality and health.

*Arabidopsis halleri* (L.) O’Kane & Al Shehbaz, is one of the two species known to hyperaccumulate Cd (Küpper et al., 2000). *A. halleri* is also a Zn hyperaccumulator and usually occurs on Zn, Cd and Pb contaminated sites. Interestingly, it is closely related to and interfertile with *Arabidopsis lyrata* ssp. *petraea* (L.) O’Kane & Al Shehbaz that is both non-tolerant and a non-accumulator. Using the F2 derived from the cross between *A. halleri* and *A. lyrata* ssp. *petraea*, Macnair et al. (1999) showed that Zn tolerance and Zn hyperaccumulation were genetically independent characters. On the contrary, cadmium tolerance and hyperaccumulation were apparently correlated in *Thlaspi caerulescens* (Escarré et al., 2000; Lombi et al., 2000). Until now, the genetics of cadmium tolerance and hyperaccumulation in *Arabidopsis halleri* has not been investigated.

This work represents a first step in the analysis of the genetics of Cd tolerance and Cd hyperaccumulation in *A. halleri*, using a backcross progeny derived from the cross between *A. halleri* and *A. lyrata* ssp. *petraea*. The study was conducted in hydroponic culture. The backcross progeny segregated for both Cd tolerance and accumulation. The following questions were addressed: What are the genetic bases of Cd tolerance and hyperaccumulation in *A. halleri*? Are Cd tolerance and Cd hyperaccumulation related characters? Are Zn and Cd tolerance correlated characters? What is the relationship between Cd accumulation and Zn accumulation? The results support that i) Cd tolerance is a more complex character than Zn tolerance in *A. halleri*, that may be governed by more than one major gene, ii) Cd accumulation is a complex character, iii) Cd tolerance and Cd accumulation are independent characters, iii) Cd and Zn tolerances are related characters and iiiii) Cd and Zn are co-accumulated.


Thlaspi caerulescens EST sequence analysis: a pilot experiment

Mark G.M. Aarts¹, Peter Goossens², Martijn Fiers², Ana Assunção³ and Henk Schat³.

¹Lab of Genetics, Wageningen University, Postbus 9101, 6700 HA Wageningen, The Netherlands; ²Plant Research International, Wageningen, The Netherlands; ³Dept. of Plant Ecology and Ecotoxicology, Vrije Universiteit, Amsterdam, The Netherlands.

Thlaspi caerulescens is a well-studied natural heavy metal hyperaccumulating species, of which several accessions are known. The accession “La Calamine” (LC) originates from a calamine ore waste in Belgium, and is highly tolerant to and accumulating zinc and cadmium. Accession “Monte Prinzena” (MP), originating from serpentine soils in Italy, is adapted to nickel exposure, whereas accession “Lellingen” (LE) was collected from non-metalliferous soil in Luxembourg. The genetic origin underlying and controlling heavy metal hyperaccumulation in plants is still largely unknown, but T. caerulescens appears to be a suitable species to study this phenomenon conveniently. Reciprocal crosses have been made between these accessions and F3 populations are currently constructed and phenotyped. These populations will provide valuable material for the identification of intraspecific genetic variation contributing to zinc, cadmium or nickel tolerance and accumulation.

In a previous study (Assunção et al., 2001), we identified three different metal transporters in T. caerulescens, with homologies to the ZIP and ZAT-type of metal transporters identified in Arabidopsis thaliana. These three genes were much higher expressed, even under elevated zinc-exposure conditions, in T. caerulescens, compared to the expression of their orthologues in the non-accumulating T. arvense and A. thaliana. This prompted us to see if there were other T. caerulescens cDNAs which were expressed at higher levels compared to their orthologues. In a pilot experiment, 106 random cDNA clones were picked from a LC root library (grown at 10 µM Zn²⁺) and their partial 5’ DNA sequence was determined. The in silico analysis of these sequences will be presented and discussed.
DIFFERENTIAL EXPRESSION OF GENES INVOLVED IN ZINC TOLERANCE

*Arabidopsis halleri* is both a zinc resistant and zinc hyperaccumulating plant. In hydropony, *A. halleri* roots continue to grow at zinc concentration over 2mM, in strong contrast with the sensitive, non-accumulating relative *Arabidopsis lyrata* spp. *petreae* which roots stop to grow at c.a. 50 µM zinc. This is obviously a strong, contrasting adaptive phenotype of zinc resistance. The same hold true for accumulation where zinc accumulates up to several % DW in the aerial part of *A. halleri*, whilst its accumulation is rapidly stopped by toxicity in *A. lyrata*. Crosses with *A. lyrata* have allowed to genetically characterize the single dominant locus for zinc resistance in *A. halleri*, which is independent from the hyperaccumulation trait (Macnair et al., 1999).

Despite of having a different number of chromosomes (2n=10 for *A. thaliana*; 2n=16 for *A. lyrata* and *A. halleri*), preliminary analysis show a high level of synteny between *A. lyrata* and *A. thaliana* (Savolainen O., personal communication). In addition: (i) fertile interspecific hybrids can be obtained in the cross between *A. lyrata* as the male parent and *A. thaliana* as the female parent (Nasrallah et al., 2000) and (ii) *A. halleri* can be used as the male parent in crosses with *A. lyrata* to generate fertile hybrids.

Suppression-subtractive hybridization (SSH) is a powerful tool to reveal the differentially expressed genome part between two conditions. We reasoned that the ideal differential situation would be created by pooling the opposite phenotypes in the segregating progeny issued from the cross between two parents with extreme opposite phenotype. C.a. 300 BC1 individuals from the crosses between F1 (*A. halleri* x *A. lyrata*) and *A. lyrata* have been genotyped and then phenotyped for zinc resistance (and are actually phenotyped for zinc accumulation). Plants have been grouped in phenotypic categories with the same level of resistance. Two extreme groups were observed and grouped by pools of 20 randomly chosen plant in each extreme phenotypic class giving rise to a sensible pool and a resistant pool, thus creating bulk segregants. These individual can be maintained because of the capacity of these plants to be propagated by cuttings. Expectation is that there is a statistical homogenization of the expressed genome between the two pools except for what has been selected between them, i.e.: resistance and accumulation. This bulk segregants analysis represents a differential situation that we hypothesized maximizes the likelihood to pick up differentially expressed cDNA specific for the selected phenotype. This is expected to increase the signal/noise ration during the construction of the substractive library by SSH.

Expression of various cDNAs in the yeast *Saccharomyces cerevisiae* is a well demonstrated way to assess rapidly the tolerance functionality of candidate genes isolated by SSH, as well as to directly screen for the cDNA from an *A. halleri* leaf cDNA library that could confer resistance to lethal concentration of zinc in the medium. This yeast transcriptome functional approach complement the SSH approach and has the advantage to directly provide the cDNA with the desired phenotype associated.


Genomic structure analysis by AP-PCR of three plant species growing around an abandoned Antimony mine

Elen Jones-Evans, Elena Maestri, Nelson Marmiroli

Division of Genetics and Environmental Biotechnology, Department of Environmental Sciences, University of Parma, Parco Area delle Scienze, 43100 Parma, ITALY

Antimony is a trace element, non-essential to plants. However it is readily taken up by roots from soils when in soluble form and if it occurs in large quantities can be phytotoxic. Contaminated areas exert high selection pressures on the species found there, favouring the evolution of endemic plant species or ecotypes of species that normally grow on non-contaminated soil, capable of growth and reproduction in toxic and often nutrient-poor environments. Indeed some plant species accumulate metals up to high levels without exhibiting phytotoxicity. In this work, the genetic variability within and among populations of three perennial herbs, *Plantago lanceolata*, *Achillea ageratum* and *Inula viscosa*, is studied; the former two previously reported as being antimony accumulators. The chosen site is an abandoned antimony mine situated in the Monti Romani area, Southern Tuscany, Italy. Plant populations from three areas around the mine (control-low, intermediate and high contamination levels) were sampled and analysed using molecular markers generated by the Arbitrarily Primed (AP) PCR technique. Twenty primers (20 nt ca.) were designed on conserved regions of genes induced or involved in heavy metal resistance, using the tomato (*Lycopersicon esculentum*) and *Arabidopsis* gene sequences for primer design. These genes being gamma-glutamylcysteine synthetase, glutathione synthase, phytochelatin synthase and metallothioneins from groups 1 and 2.

By designing primers on specific genes, a fingerprint of amplified anonymous fragments enriched in fragments corresponding to coding sequences is generated. Principal Coordinates Analysis on the matrix of genetic distances among pairs of individuals indicates that individuals from the same population cluster together. Preliminary results evidence that genetic diversity is significantly higher in populations from areas at intermediate and high levels of contamination. The proportion of variance apportioned to differentiation among populations is not high, but significant. As the molecular markers evidenced by AP-PCR are gene targeted, they will be subject to selection; therefore valuable information on the biological resources available at a given site can be obtained.
Serial Analysis of Gene Expression (SAGE) : sequencing-based method for large scale transcript profiling in *Arabidopsis*.

Stéphane Muños.
Laboratoire de Biochimie et Physiologie Moléculaire des Plantes, UMR 5004, Agro-M, CNRS, INRA, UM-II, Place Viala, 34060 Montpellier Cedex 1, France.
e-mail : munos@ensam.inra.fr

Since the beginning of the sequencing program of the model plant *Arabidopsis thaliana*, new biological and computer tools have permitted an accumulation of more and more data. Now, the complete sequence of the five chromosomes constituting the *Arabidopsis* genome is available and the sequencing of other genome's species (Rice, Medicago...) has yet begun. This large amount of sequence data is analysed by computers in order to identify genes and when possible their function. However, functions assigned by these algorithm-based methods, without experimental basis, are not safe and only putative. A new area has emerged : "Functional Genomic" which has the ambitious aim to experimentally determine the function and the regulation of all genes. High throughput methods have been developed to characterize the patterns of expression of a large number of genes. The first one was based on the sequencing of partial cDNA (Expressed Sequence Tags) but do not permit to compare the expression level of genes between several conditions. New methods, based on hybridization, using DNA chips or macro-arrays have been developed to monitor the expression of thousand of genes (transcriptome).

We report here the successful use on *Arabidopsis* of an alternative method for obtaining transcriptomes, called SAGE (Serial Analysis of Gene Expression). This sequencing-based method, already used on animals and yeast, has only been marginally exploited with plants (Matsumura *et al.*, 1999). First described by Velculescu *et al.* (1995), SAGE allows the quantification of the respective levels of expression of thousand genes. Detailed description of the method will be presented and advantages/disadvantages as compared to other methods will be discussed. Results from SAGE transcriptomes obtained using an *Arabidopsis* nitrate transport mutant will be presented to demonstrate the ability of the technique to identify genes differentially expressed between genotypes or environmental conditions.

References :
Genome-wide approaches in the attempt to understand the molecular basis of metal hyperaccumulation in *Arabidopsis halleri*

Ute Krämer, Martina Becher, Dörthe Dräger and Rhonda Meyer  
Max Planck Institute of Molecular Plant Physiology, 14424 Potsdam  
ute@mail.com

*Arabidopsis halleri* (accession Langelsheim) is a Zn hyperaccumulator. Compared with *A. thaliana* (accession Columbia) *A. halleri* also displays an elevated tolerance to the metals Zn and Cd. In order to identify candidate genes which might function in either hyperaccumulation or metal tolerance we are pursuing several genome-wide approaches, screening for either specific functions of the gene products or differential gene expression at the transcript level. In a functional screening approach we have obtained a size-fractioned cDNA library, which has been cloned into the yeast expression vector pYES2. Phenotypic screens have been designed to identify candidate cDNAs upon heterologous expression in yeast. In our expression profiling screening approaches we are using two strategies: cDNA AFLP and microarray hybridisation (AFFYMETRIX™). These approaches will be presented, and preliminary results will be discussed.
ISOLATION, CHARACTERIZATION AND ANALYSIS WITH ADVANCED PHYSICAL TECHNIQUES OF ARABIDOPSIS THALIANA MUTANTS OBTAINED BY “T-DNA TAGGING” RESISTANT TO CAESIUM

Marmiroli M.¹, Visioli G.¹, De Bastiani M.¹, Antonioli G.², Maestri E.¹, Marmiroli N.¹
¹ Dip. Scienze Ambientali, Università degli Studi di Parma; Tel 0521905687, Fax 0521 905665; e-mail: maestri@unipr.it ² Dip. di Fisica, Università degli Studi di Parma

Arabidopsis thaliana T-DNA insertion lines have been utilized for an extensive study on tolerance to Caesium ions, in the natural stable form, in synthetic media added with different Potassium concentrations. It has been demonstrated that sensitivity to Cs depends on K concentration. Plants which were able to grow in the presence of Cs doses of 600 µM, inhibiting growth of wild type plants, have been selected by screening at least 1200 seeds from each of 49 pools of 100 T-DNA insertion lines. PCR-amplification of genomic DNA from surviving plants confirmed the presence of T-DNA fragments. In this way, 10 Cs-resistant lines have been confirmed. Two of these plants, “a063c” and “a171f”, have been brought to seed setting in order to perform genetic and molecular analysis in the following generations. In order to characterize the physiological mechanism of Cs resistance in these mutants, two technologies have been applied for measuring Cs intake: synchrotron-radiation-induced X-ray microfluorescence (µ-SRXF), carried out at the LURE laboratories (France), and SEM/EDX microanalysis, carried out at the University of Parma. The data obtained by comparing sensitive and resistant plants through µ-SRXF are summarized in Table I (cps = counts per second of X-ray photons emitted by the sample).

<table>
<thead>
<tr>
<th></th>
<th>w.t.</th>
<th>a171f</th>
<th>a063c</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>55000 cps</td>
<td>7000 cps</td>
<td>9000 cps</td>
</tr>
<tr>
<td>Ca</td>
<td>20000 cps</td>
<td>4500 cps</td>
<td>6000 cps</td>
</tr>
<tr>
<td>Cs</td>
<td>12000 cps</td>
<td>500 cps</td>
<td>450 cps</td>
</tr>
<tr>
<td>K/Cs</td>
<td>4.5</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>K/Ca</td>
<td>2.75</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca/Cs</td>
<td>1.6</td>
<td>9.0</td>
<td>13.3</td>
</tr>
</tbody>
</table>

In both mutants, Cs and K intake is reduced, and the K/Cs ratio in resistant mutants is higher than in wild type plants. Similarly, a reduction in Ca intake can be evidenced, without a corresponding modification of the K/Ca ratio in sensitive and resistant plants. Therefore, in resistant plants the mutation leads to a reduction in intake for different ions (K, Ca, Cs) with more pronounced effects on Cs intake. This effect could be attributed to the existence of specific ion channels for Cs, which could however be utilized less efficiently and not exclusively also for Ca and K intake. Genetic analysis is currently in progress by exploiting T-DNA tagging of the two mutants.
Postgenomic tools for the analysis of metal tolerance and accumulation in the model plant *Arabidopsis halleri* and its relative *Arabidopsis thaliana*

Stephan Clemens, Christoph Vess, Michael Weber, Thomas Degenkolb, Udo Roth, Edda v. Roepenack-Lahaye

Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale), Tel. 49-345-5582-1420, Fax 49-345-5582-1409, email: sclemens@ipb-halle.de

In order to biochemically monitor plant metabolic changes in response to environmental changes we established the profiling of proteins, peptides and secondary metabolites in *Arabidopsis thaliana*. Challenge of hydroponically grown plants with heavy metals such as cadmium serves as our model stress condition. Metabolites are analyzed by GC-MS and LC-ESI-Q-TOF-MS of methanolic extracts of roots and leaves. Proteins are detected following 2D-PAGE by MALDI-TOF-MS and tandem-MS of tryptic digests. MS-based searches for putative signalling molecules are currently focussing on the intercellular washing fluid sampled from leaves of treated plants.

Techniques and data established for *A. thaliana* will also be used for the analysis of the metallophyte *A. halleri*, which is a Zn hyperaccumulator and more Cd tolerant than *A. thaliana*. 2D-PAGE of root extracts has been optimized and protein identification is currently being tested by direct sequencing of peptides via ESI-Q-TOF-MS-MS.
Functional genomics of *A. thaliana* gene families involved in detoxification

**Thulke, O.**\(^1\), Glombitza, S.\(^1\), Messner, B.\(^1\), Geist, B.\(^1\), Welzl, G.\(^2\), Bovet, L.\(^3\), Martinoia, E.\(^3\), Hehn, A.\(^4\), Werck-Reichhart, D.\(^4\), Mauch, F.\(^5\), Schäffner, A.R.\(^1\)

\(^1\)Institute of Biochemical Plant Pathology and \(^2\)Institute of Biomathematics, GSF Research Center for Environment and Health, D-85764 Neuherberg, Germany, \(^3\) Laboratoire de Physiologie Végétale, Institut de Botanique, University of Neuchâtel, CH-2007 Neuchâtel, Switzerland, \(^4\)IPMB-Strasbourg, F-67084 Strasbourg Cedex, France, \(^5\)Institute de Biologie Vegetale, Universite de Fribourg, CH-1700 Fribourg, Switzerland

Detoxification of xenobiotics in plants involves many different enzymes usually classified as phase I, II, and III activities. These include cytochrome P450 monooxygenases (CYP), glutathione S-transferases (GST), glycosyltransferases (UGT) or ABC-type transporters (ABC). The availability of the first whole plant genome sequence of *Arabidopsis thaliana* revealed an unforeseen complexity and diversity of these enzyme classes. In order to identify individual candidate members that play a role in detoxification we are using DNA array analyses to monitor transcriptional changes of CYP, GST, GT and ABC in response to herbicides sprayed at sublethal doses. Treatment with bromoxynil led to the induction of a specific subset of genes that was clearly distinct from genes induced by two different sulfonylureas. Only a few genes were induced by both herbicide classes. The focus in our research group is on UGTs. Further functional analyses of candidate genes will employ transgenic overexpression and repression of individual UGTs identified by expression profiling. Transgenic plants will be analysed for altered sensitivity to xenobiotics and other environmental stresses. Interestingly, recombinantly expressed UGT-23, that is specifically induced by bromoxynil, is able to glucosylate 3,5-dibromo-4-hydroxy benzoic acid, a primary hydrolysis product of bromoxynil.
Exploring the Glutathione Transferase Xenome

David P. Dixon and Robert Edwards

School of Biological & Biomedical Sciences, University of Durham, Durham DH1 3LE UK

Just as individual plant species have a unique ability to metabolise and accumulate endogenous natural products, the so-called metabolome of the plant, each species also has a unique ability to biotransform xenobiotics, which we term its xenome. The plant xenome is derived from a unique collection of enzymes of secondary metabolism, notably the detoxification systems making up the three phases of drug metabolism. Recently, valuable insights into the genetic diversity of plant xenomes has been obtained from examining the gene families encoding mixed function oxidases, glutathione transferases, glucosyltransferases and ABC transporters which have been obtained from EST and genome projects in different species.

The glutathione transferases (GSTs) represent a useful model system for applying the study of genomics to xenome biology as:

1) GSTs already have a well defined role in the detoxification of xenobiotics in plants
2) The patterns of expression of GSTs are known to be species specific, as this forms the basis of the selectivity of several major herbicides
3) The GST gene family is not too large as to defy comprehensive analysis. Furthermore, within the 4 classes described to date only the phi and tau classes are active in xenobiotic metabolism.

For any given plant, the GST xenome will be defined by:

1) The diversity of phi and tau class GSTs present.
2) The regulation of their expression
3) The intimate interaction with other xenome enzymes, such as mixed function oxidases involved in supplying GST substrates and ABC transporters involved in removing GST reaction products.

Using examples from recent studies, we will review recent progress in understanding the GST xenome in maize and soybean, where GSTs have been studied at both the biochemical and molecular level in planta. Using Arabidopsis we will then consider how genome information may be useful in addressing questions of GST xenome biology in other plants in the future and how this information could be of use in phytoremediation.
Addressing Proteomic Approaches in Phytoremediation

Richard P Haslam\textsuperscript{A}, Karine Gallardo\textsuperscript{B}, Dominique Job\textsuperscript{B} and Julian O D Coleman\textsuperscript{C}.

\textsuperscript{A} Crop Performance and Improvement, IACR-Rothamsted, Harpenden, AL5 2JQ, United Kingdom.
\textsuperscript{B} Laboratoire Mixte Centre National de la Recherche Scientifique-Institut National de la Recherche Agronomique-Aventis, Aventis CropScience, Lyon, France.
\textsuperscript{C} School of Biological & Molecular Sciences, Oxford Brookes University, Oxford, OX3 OBP, United Kingdom.

Classical experimental approaches have used biochemical, physiological and structural studies to elucidate plant responses to the wide range of xenobiotics they encounter. All of these approaches yield results relevant to the problem but tend to focus on particular components and do not give a complete representation of how the cells respond. In view of the recent completion of the sequencing of the first plant genome proteomics has emerged as a strategy to examine the global response of cells to environmental challenges. At the same time the term “proteome” was conceptualised to define the expressed complement of a genome. Initial studies have concentrated on the construction of proteomes from complex origins, such as the rice shoot proteome, in most cases this kind of descriptive proteomics has now gained a functional dimension by focusing on specific subcellular proteomes.

The localisation of proteins in the apoplast (one of the first points of contact between the plant and the environment) capable of metabolising xenobiotics will consequently influence the whole plant uptake and toxicity of any compound. As a discrete compartment, the leaf apoplast contains an array of proteins and enzymes involved in plant response to xenobiotic challenge. Despite the availability of abundant sequence information from \emph{Arabidopsis} in the databases, it is difficult to clearly identify those proteins with a specific apoplastic location. Therefore, the application of a proteomic strategy represents a unique opportunity to characterise the contents, and therefore the role, of the apoplast proteome. In the present study apoplast proteins were extracted from \emph{Triticum aestivum} and \emph{Arabidopsis thaliana} by vacuum infiltration followed by gentle centrifugation. Extracted apoplast and total proteins were then resolved by 2-D gel electrophoresis, prior to silver and Coomassie staining. A systematic comparison of the 2D gels with total protein extracts allowed classification of proteins to the apoplast proteome. A selection of the proteins characteristic of the apoplast in \emph{A. thaliana} were excised from gels, digested with trypsin, and analyzed by matrix assisted laser-desorption ionization time of flight (MALDI-TOF) mass spectrometry. The aim of this work was I) generate protein 2D maps of the apoplast from different species II) reveal unexpected locations for proteins characterised elsewhere in the cell, and III) obtain sequence information on apoplast specific xenobiotic metabolising proteins. The implications for phytoremediation and the technical difficulties associated with such a holistic approach to protein discovery will be discussed.
Flexibility of detoxification enzymes in Cattail (Typha spec.)

Christian Scheer\textsuperscript{1}, Beate Huber\textsuperscript{1}, Anita Rudy\textsuperscript{1}, Otto Theobald\textsuperscript{2} and Peter Schröder\textsuperscript{1}

\textsuperscript{1}Institut of Soil Ecology, GSF-National Research Center for Environment and Health, D-85674 Neuherberg, Germany
\textsuperscript{2}IFUWA-GmbH, Institut für Umweltschutz, Wasser, Altlasten, und Geotechnik, D-85051 Ingolstadt, Germany

In biological sewage plants, swamp plants are mostly utilized to filter out nutrients, such as phosphate and nitrate. However xenobiotics and drugs occur in sewages in substantial concentrations as well. It is of interest therefore to explore whether these swamp plants are also able to degrade xenobiotics and drugs in considerable amounts. Due to the strengthening of the water directive of the European Community the need to improve and maintain high quality standards for sewage treatment effluents during the next years is of importance. Plant-based treatment systems may offer an adequate supplement to existing technologies.

\textit{Thypha latifolia} (L.) and \textit{Typha angustifolia} (L.) were investigated for a) the general detoxification capacity of organic xenobiotics; and b) the fate and specific breakdown of two persistent substances: bis (2-ethylhexyl)-phthalate (DEHP) (a plasticizer) and Lamotrigine (an antiepileptical medicament). These are substances of high environmental concern.

Preliminary results indicate that Typha plants possess peroxidase activity and glutathione S-transferase (GST) activity for the conjugation of several xenobiotic model substrates (i.e. CDNB, DCNB etc.) in leaves, rhizomes and roots.

Total GST activity seems to be shared by several GST-isoforms. Whereas the activity of some Glutathione S-transferase remains unaffected following the application of xenobiotics in induction experiments, other are induces by both chemicals and medicaments.

In studies with Typha roots and rhizomes, the removal DEHP and Lamotrigine was observed. This disappearance seems to be connected to the activity of GSTs or Glucosyltransferases. The significance of this effect for the utilisation of Typha in sewage treatment is discussed. Further studies using contaminated wastewater are planned.
GENOMICS OF PLANT GSTS

C. Frova, L. Mizzi, N. Soranzo, M. Sari Gorla
Department of Genetics and Microbiology, University of Milano, Via Celoria 26, 20133 Milano, Italy

Glutathione S-transferases (GSTs) are superfamily of proteins present with several members in all eukaryotes. They have evolved independently in different organisms, giving rise to a variety of catalytic and non-catalytic roles, all associated with the defence against toxic molecules and environmental stress in general. Recent evidence suggests that plant GSTs encompass over 40 members per species, belonging to four structurally diverse classes, the functional diversity of which continues to be discovered. Besides being important in herbicide detoxification, via conjugation with glutathione and vacuolar compartmentation, they have been shown to act as glutathione peroxidases, esterases, isomerases and to have roles in stress induced signalling and preventing apoptosis. Indeed GST activity is induced in response to several insults of biotic and abiotic origin, among which pathogen attack, bioactive xenobiotics and oxidative stress caused by various sources, including heavy metals. However, sequence-structure-function relationships within the GST family are still pretty obscure.

We are currently investigating the genomic organisation of GSTs in different monocotyledonous and dicotyledonous species, particularly in the two model species rice and Arabidopsis. A large number of genes has been identified for the two species within sequence databases and EST collections. Their genomic structure and evolutionary relationships are investigated using bioinformatic tools, with the future aim to correlate evolutionary closeness among members of the family and their expression patterns in response to various sources of chemical stress.
Potential of desert plants as heavy metals hyperaccumulators

Golan-Goldhirsh, A., Manandhar, U., Yakubov, B and Vulkan, R.

In this project for the first time desert plants of the Negev were screened and evaluated for their heavy metal tolerance/hyperaccumulation. The initial screening was done at the whole plant level by measurement of heavy metal (Cu, Zn, Ni, Cd, Co and Pb) content. Selected plants, *Nicotiana glauca*, *Atriplex halimus*, *Kochia indica*, *Mercurialis annua* and *Mesembryanthemum nodiflorum*, which showed higher heavy metals accumulation in the natural conditions were tested further under controlled nutritional conditions. The results led to a list of potential hyper-accumulators (accumulation in shoot), *Mesembryanthemum nodiflorum* for Cu, Pb, Ni, and Cd; *Mercurialis annua* for Ni and Cd; *Nicotiana glauca* for Cd and Co; and *Atriplex halimus* and *Kochia indica* for Cd. All desert plants tested, accumulated high concentrations of heavy metals in their roots. The usefulness of this property for practical phytoremediation is discussed. It raises interesting questions regarding the mechanisms of heavy metal tolerance in root and avoidance of translocation of the metals to the shoot.

Evaluation of desert plants growth on heavy metal contaminated soil from a solid waste site showed a general pattern in which *Atriplex halimus*, *Kochia indica* and *Mesembryanthemum nodiflorum* tolerated the contaminated soil without obvious morphological symptoms. However, for the duration of the experiment (4 weeks) the accumulation of Zn and Cu in the plants did not reach hyperaccumulation levels. The effect of addition of EDTA and the combination of EDTA and compost enhanced the availability and uptake of the metals from the soil. However, compost alone decreased the concentration of metal in the soil water soluble fraction, which leads to the conclusion that it is the EDTA that increases availability of heavy metals to the plants.

PC synthase (PCS) from *Atriplex halimus*, *Mesembryanthemum nodiflorum* and *Mercurialis annua* was cloned and partially sequenced. There was high sequence homology among the desert plants, as well as to PCS of other species, indicative of a highly conserved gene.
Identification of Xenobiotic Metabolising Proteins from Rice using Proteomic Approaches.

Andrew L downie\textsuperscript{A} Richard P Haslam\textsuperscript{A}, Karine Gallardo\textsuperscript{B}, Dominique Job\textsuperscript{B} and Julian O D Coleman\textsuperscript{C}.

\textsuperscript{A} Crop Performance and Improvement, IACR-Rothamsted, Harpenden, AL5 2JQ, United Kingdom.
\textsuperscript{B} Laboratoire Mixte Centre National de la Recherche Scientifique-Institut National de la Recherche Agronomique-Aventis, Aventis CropScience, Lyon, France.
\textsuperscript{C} School of Biological & Molecular Sciences, Oxford Brookes University, Oxford, OX3 OBP, United Kingdom.

The aim of the present project is to identify apoplast specific xenobiotic metabolising proteins from rice (\textit{Oryza sativa}). Rice is an important model species for monocotyledonous plants, especially for members of the graminacous family. It is an attractive system for study largely because of its small genome, estimated to be 430 Mb, and also because of its diploid nature, transformability and the fact that genetic and molecular resources are now well established. International efforts have succeeded in sequencing the entire genome of \textit{O. sativa} spp. \textit{Japonica} var “Nipponbare” using a bacterial artificial chromosome shotgun sequencing strategy. Rice is the first major cereal crop for which sequence data has become available, and is therefore an ideal system in which to investigate plant xenobiochemistry.

The apoplast is potentially the first biochemical compartment of the cell to be encountered by a xenobiotic, and it is here that relevant detoxifying proteins could be located. A method has been optimised for the extraction of apoplastic fluid from rice leaves, using a vacuum infiltration approach.

The protein mixture, which was free from cytoplasmic contamination, was resolved using high-resolution two-dimensional polyacrylamide electrophoresis (2-D PAGE), prior to Coomassie blue or silver staining. Total-leaf extracts were subjected to identical treatments, and comparison of the two protein maps revealed proteins likely to reside specifically in the apoplast. The most abundant apoplast proteins were excised from the gel and characterised after trypsin cleavage by matrix assisted laser desorption-ionization time of flight (MALDI-TOF) mass spectrometry and peptide mass fingerprinting.

The identification of proteins capable of detoxifying xenobiotics has clear implications for phytoremediation, and these will be discussed.
PHYTAC – Development of systems to improve phytoremediation of metal contaminated soils through improved phytoaccumulation

Kärenlampi S¹, Tervahauta A¹, Hassinen V¹, Koistinen K¹, Aarts M², Koornneef M², Atanassov A³, Gorinova N³, Nedkovska M³, Todorovska E³, Iordanova E³, Schat H⁴, Ernst WHO⁴, Assuncao A⁴, Jack E⁵, Herzig R⁵, Rehnert A⁵, Erismann K-H⁵, Vangronsveld J⁶, van der Lelie D⁶, Ernst D⁷, Sandermann H⁷, Knabel S⁷
¹University of Kuopio, Finland; ²Wageningen University, The Netherlands; ³Institute of Genetic Engineering, Bulgaria; ⁴Vrije Universiteit Amsterdam, The Netherlands; ⁵Arbeitsgemeinschaft für Bioindikation, Umweltbeobachtung und ökologische Planung, Switzerland; ⁶Limburgs Universitair Centrum, Belgium; ⁷GSF-Institut für Biochemische Pflanzenpathologie, Germany

The present state of knowledge about the mechanisms of metal tolerance, uptake and accumulation is fairly limited and does not support particularly well the construction of designer plants for remediation. There is thus a need to develop this knowledge. Strong emphasis is placed in the new EU V Framework project PHYTAC on the exploitation of technologies such as genomics and proteomics to identify metal responsive genes and proteins in metal hyperaccumulator plants. The expected deliverable is a toolbox of genes that can be used to improve tolerance of plant to metals as well as metal accumulation and transport in the plant. These tools will contribute to efforts being made to optimise systems for phytoremediation eventually suitable for commercial exploitation.