

# QTL mapping and the tools of molecular evolution to validate genes potentially responsible for heavy metal tolerance in *Arabidopsis halleri*

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## INTRODUCTION

The control of intracellular metal concentrations by plants has several important implications for human health. Increases in Zn, an essential nutrient, content of edible parts of crops increases their nutritive quality, but the accumulation of Cd, a toxic metal, by crops is the biggest cause of Cd ingestion by humans. As a result of their intrinsic immobility, plants have evolved a wide range of strategies to face adverse environments, including toxic levels of metals. Numerous reports show that plant resistance to trace metals is a complex mechanism that relies on the control of uptake, exclusion, translocation, compartmentation and sequestration (Briat and Lebrun 1999). Among tolerant plant species, a rare class is able to accumulate very high amounts of trace metals in their aerial parts. Such plants have been called hyperaccumulators (Baker 1981) and have recently received considerable interest because of their potential use in phytoremediation.

The data generated by recent molecular approaches have provided clues to the basis of metal homeostasis (Becher et al. 2003, Weber et al. 2004, Bernard et al. 2004, Roosens et al. 2004). These techniques of gene screening among species that are separated by millions of years of evolutionary divergence were highly powerful in identifying differences in expression and structure for a large number of genes, but they failed to pinpoint which genes are the real cause of tolerance and hyperaccumulation and what are the consequences of the selection of the traits.

Because recent mapping in *A. halleri* confirmed high synteny with *A. thaliana* (Willems et al. submitted), we propose to (i) select candidate genes (isolated by "traditional" screening) according to their theoretical presence in quantitative trait loci (QTLs) of Zn/Cd tolerance in *A. halleri*; (ii) validate their position by mapping, and (iii) test which candidate genes "validated" for their linkage disequilibrium with one of the traits could be involved in the metal tolerance process by studying their sequence polymorphisms. Selective sweeps will be identified by comparing gene sequences among different species (tolerant and non-tolerant) of the *Arabidopsis* genus. This multidisciplinary approach will offer new perspectives to study molecular evolutionary processes responsible for metal tolerance.

## METHODS

The main genes responsible for Cd and Zn tolerance were studied using the following steps: Candidate genes were validated by their presence in the QTL regions for Zn and/or Cd tolerance detected in a segregating progeny produced by an inter-specific cross between *A. halleri* and *A. lyrata petraea*. The following methodological approach was developed to perform the mapping: conserved primer pairs were used for amplified homologous fragments from *A. halleri* and *A. lyrata*. Sequence variation between the parents was investigated to allow a detection of homozygote and heterozygote genotypes in the BC1 progeny and thereby the mapping of the genes.

The nucleotide polymorphisms of alleles at the gene loci in the Cd/Zn tolerance QTLs were compared between tolerant and non-tolerant *Arabidopsis*.

## RESULTS AND DISCUSSION

Interspecific crosses between *A. halleri* and *A. lyrata*, led to the mapping of three QTL regions for Zn and Cd tolerance (Willems et al. submitted, Courbot et al. submitted). Taking into account the good macro-synteny between *A. thaliana* and *A. halleri*, the positions of the QTL regions of Zn/Cd tolerance identified in *A. halleri* were localised on the *A. thaliana* genome. In the first step, the position of several candidate genes were analysed *in silico* in *A. thaliana*, and those which are localised in the theoretical QTL regions were selected. In the second step, the selected genes were mapped in *A. halleri* to assess whether they are in the QTLs of Zn /Cd tolerance.

At the present time, major candidate genes that co-localize with the pic of each QTL were selected and their nucleotide sequence will be determined in populations of *A. halleri* (tolerant) and *A. lyrata* (non-tolerant). All sequences will be compared with *A. thaliana* sequences from available databases. Nucleotide polymorphisms within each of these two categories of samples will be determined and compared. Strong reductions in polymorphisms within the highly tolerant category could pinpoint genes or genomic regions that have experienced a recent fixation of an allele through directional positive selection "selective sweep"(Wang et al. 1999). Differences in polymorphisms between promoter and coding regions will allow determining if the selected mutation has targeted gene expression or gene function. Comparisons of the rate of evolution of these genes across the history of each category will be realised using assessments of divergence from *A. thaliana*. Such data could pinpoint lineages that encountered accelerated evolution under positive selection. The future objective is to study the effect of the systematic modification found in the coding sequences of these genes on the function of the proteins they encode by functional expression in yeast mutants.

## ACKNOWLEDGMENTS

This work is supported by a Marie Curie intra European fellowship (proposal 024683 METOLEVOL).

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