

Salicaceae species: identification of molecular functions and analytical descriptors involved in metal uptake and translocation

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The potential for an enormous genetic diversity of poplar and willow has suggested searching among clones of an Italian germplasm collection of these species to select material with a high capacity for phytoextraction of specific heavy metals from soils or waters. To this scope, 20 clones, selected for contrasting phenotypic characteristics, identified in previous experiments, were exposed to toxic concentration of cadmium solution. Growth and physiological parameters of exposed plants, as well as, their ability to accumulate cadmium in roots and to translocate it to leaves were determined and six genotypes with the best characteristics for phytoextraction were further selected. Data for all ecophysiological and morphological parameters were subjected to analysis of variance (ANOVA) using the SPSS software supplemented with multiple-comparison test of the means using the Tukey-Kramer method with a significance level of $P < 0.05$. We also used hierarchical cluster analysis to classify the clones based on the results of our experiment.

Results showed interesting aspects of the plant interaction with the absorbed cadmium. For example, clones with high net photosynthesis had higher total dry mass and cadmium content. Chlorophyll content was not related to the maintenance of a high photosynthetic capacity. It was also shown that *salix* can absorb per plant more cadmium than *poplars* and maintain it in roots up to 50% with respect to the 80% maintained in poplars. By means of statistical analysis clones were ranked for specific phytoextraction abilities including tolerance variation towards the amount of cadmium absorbed in roots or translocated to shoots.

The six selected genotypes are also under genetic analysis by selecting genes involved in the different steps of the process: cadmium uptake, sequestration, compartmentalisation, and translocation. A database has been developing for *Populus* species containing EST homologous to genes isolated from different plant species. The genes encode for proteins involved in tolerance to heavy metals: metallothioneins (Zhou and Goldsbrough 1994); enzymes for phytochelatin synthesis (Howden et al 1995); tonoplast proteins (Salt et al, 1998); proteins produced under high temperatures and oxidative stresses (Diderjean et al 1996). Genes homologous to different transporters involved in heavy metal uptake will be also isolated from *Populus* genome (<http://genome.jgi-psf.org/Poptr1/Poptr1.home.html>). Single nucleotide polymorphisms (SNPs) will be looked for by comparing sequences from different poplar clones and by identifying those sequences that may be potentially responsible for allelic variants with functional significance. This experimental pathway will eventually help in identifying possible molecular descriptors of plants performance in phytoremediation. Two poplar clones were also investigated for mapping metal concentration within roots through scanning electron microscopy (SEM) and energy dispersive ray-x (EDX). Control plants had metal concentration below the instrument sensitivity. From SEM-EDX analysis, clone 6K3 was found to take up higher Cd amounts than clone 14P11. Higher Cd concentrations were found in the apical portion of roots in clone 6K3, while in the intermediate portion in clone 14P11. Amongst the elements considered, only Fe and P were significantly affected by metal treatment in both clones, while Cu, K and S showed significant interaction clone x treatment.

References

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