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Poster Abstract - L.02

PHYTOREMEDIATION OF CHROMIUM USING WILLOW SPECIES: CLONING ESTs AND CANDIDATE GENES INVOLVED IN Cr ACCUMULATION

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Phytoremediation, the use of plants for environmental cleanup, has gained acceptance in the past ten years as a cost-effective, non-invasive alternative or complementary technology for engineering-based remediation methods. Plants can be used for pollutant stabilization, extraction, degradation, or volatilization. To further enhance the efficiency of phytoremediation, there is a need for better knowledge of the genetic basis and the metabolic processes that control and affect pollutant accumulation, translocation, chelation, degradation and volatilization.

Four different willow species (*S. alba, S. eleagnos, Salix fragilis, Salix matsudana*) were adopted for evaluating phytoremediation approaches, including their applicability for various organic and inorganic pollutants. Species of the genus *Salix* are known to be suitable for use in phytoremediation because of their fast growth, high biomass production and adaptability to wetland systems.

Molecular differential screening of plants with contrasting accumulation efficiency of pollutants is theoretically one of the most powerful tools for identifying and isolating genes underlying the metabolic processes of pollutant accumulation. Even in remarkably complex genomes like that of willow, a differential display based on the detection of cDNA-AFLP markers in combination with developmentally staged organs may be effective for detecting expressed sequence tags (ESTs) and cloning candidate genes responsible for or involved in chromium accumulation. In the area of phytoremediation research, such a method relies on synthesizing cDNA subsets from roots and leaves at specific developmental stages of individuals sharing the same genetic background but subjected to antagonist growing conditions, and then screening for differentially expressed transcripts between treated samples and controls. This approach makes possible the assay of a large set of mRNA-related fragments and increases the reliability of amplification-based transcriptome analysis.

The differential display in the willow species was based on the detection of restricted-ligated double-stranded cDNA fragments by PCR amplification with different AFLP primer combinations previously selected for their ability to generate informative mRNA fingerprints. The cDNA-AFLP method retrieved useful information on gene expression levels and on changes potentially related to the chromium accumulation. Various expression patterns were detected across all stages of root and leaf samples. The vast majority of transcript-derived fragments were shared among samples within species and thus attributable to constitutively expressed genes, but a number of differentially expressed mRNAs were scored in each species. In particular, upregulated or downregulated genes, that is transcripts with

increasing or decreasing expression, respectively, were visualized during root and leaf development of treated samples compared to controls. All ESTs showing differential expression patterns were excised and eluted from the gel, re-amplified, subcloned and sequenced. Their characterization by the extensive use of bioinformatics is currently in progress in order to search for structural homologies and significant similarities in public nucleotide and amino acid sequence databases.