

Gene Expression of *Medicago sativa* Inoculated with *Sinorhizobium meliloti* as Modulated by the Xenobiotics Cadmium and Fluoranthene

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Alfalfa plants (*Medicago sativa* cv. Europe) inoculated with *Sinorhizobium meliloti* 2011 (formerly *Rhizobium meliloti*, de Lajudie *et al.*, 1994) were cultivated for 14 days under standardized growth conditions in mineral medium with addition of the heavy metal cadmium or the polycyclic aromatic hydrocarbon fluoranthene. These xenobiotics significantly reduced the numbers of root nodules before any visible damage to the plant could be detected. EC10, EC50, and EC90 (effective concentrations reducing nodulation, shoot and root fresh weight by 10, 50, or 90% compared to the control without pollutant) were calculated. EC50 for cadmium ranged from 5.8 μM (nodulation) to more than 20 μM (root fresh weight). Testing fluoranthene resulted in an EC50 of 2.5 $\mu\text{g cm}^{-2}$ for nodulation, and EC50 values of more than 35 $\mu\text{g cm}^{-2}$ for shoot and root biomass production, indicating that the effect parameter nodulation is 10-fold more sensitive than shoot and root fresh weight. With mRNA differential display techniques the effects of both xenobiotics on gene expression in alfalfa root systems were studied. 37 differentially displayed transcripts were detected. Two of them, called DDMs1 and DDMs2, were confirmed by northern hybridization to be down-regulated in the presence of the xenobiotics. The expression of transcript DDMs1 was enhanced in alfalfa control plants inoculated with rhizobia, the transcript level was increased 2.5-3-fold compared to non-inoculated plants. This positive effect of nodulation was suppressed, partly by 35 $\mu\text{g cm}^{-2}$ fluoranthene and totally by 20 μM cadmium. The decrease in DDMs1 transcription was highly affected by the cadmium concentration with an EC50 of 5.9 μM . Compared to nodulation, almost identical EC10, EC50, and EC90 values were found for DDMs1 expression. Sequence analysis of DDMs1 revealed a significant overall homology (50% identity) to a hypothetical protein from *Arabidopsis thaliana* with high similarity to a copper transporting ATPase. High levels of transcript DDMs2 were observed in control plants with a 50% decrease in the xenobiotic-treated plants. DDMs2 gave a strong homology (82% identity) to the cytoplasmatic 60S ribosomal protein L18 from *Arabidopsis thaliana*.