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"Gheorghe Asachi" Technical University of lasi, Romania



## INOCULATION OF POPLAR WITH ARBUSCULAR MYCORRHIZAL FUNGI EXERTS A PROTECTIVE ROLE ON HEAVY METAL STRESS BY TRANSCRIPTOME MODULATION

## A. Cicatelli<sup>1</sup>, V. Todeschini<sup>2</sup>, G. Lingua<sup>2</sup>, P. Torrigiani<sup>3</sup>, S. Biondi<sup>4</sup>, S. Castiglione<sup>1</sup>

<sup>1</sup>Dipartimento di Chimica, Università di Salerno, 84084 Fisciano (SA), Italy; <sup>2</sup>Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale, 15121 Alessandria, Italy; <sup>3</sup>Dipartimento di Colture Arboree, Università` di Bologna, 40127 Bologna, Italy; <sup>4</sup>Dipartimento di Biologia e.s., Universita` di Bologna, 40126 Bologna, Italy

## Abstract

Phytoremediation (comprising phytoextraction, phytodegradation, rhizofiltration, phytostabilisation, phytovolatilization) is a technology that uses plants, associated or not with microrganisms, for the treatment of soil and water contaminated by organic or inorganic pollutants. Poplar is a tree species with several features suitable for phytoremediation, including a remarkable ability to tolerate high concentrations of heavy metals (HMs). Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of the vast majority of land plants; they improve nutrient uptake and can, in some cases, enhance phytoextraction and/or phytostabilisation of HMs while alleviating stress in the host plant. In a field trial on a Zn- and Cu-contaminated site, differences in plant survival and growth were observed among 168 clones originating from natural populations of *Populus* from northern Italy. One well-performing clone (AL35) had a distinctly higher concentration of both metals in the roots, and was used for investigating physiological and molecular markers associated with HM tolerance, such as metallothioneins (MTs) and polyamines (PAs). In field-grown plants, a higher concentration of the PA putrescine positively correlated with root Cu concentration. In a subsequent greenhouse study, AL35 cuttings pre-inoculated with AMF (Glomus mosseae or G. intraradices) and then transferred to pots containing soil collected from the HM-polluted site displayed growth comparable to that of controls grown on unpolluted soil, in spite of higher Cu and Zn accumulation. Such plants also showed an overall induction of MT and PA biosynthetic gene expression, together with increased free and conjugated PA levels. A genome-wide transcriptomic (cDNA-AFLP) analysis allowed the identification of a number of genes, mostly belonging to stress-related functional categories of defence and secondary metabolism, that were differentially regulated in mycorrhizal vs non mycorrhizal plants. The relevance of epigenetic changes in plant adaptation to environmental stresses was evaluated by analysing DNA methylation changes (MSAP method); on polluted soil, the most dramatic effects were observed in mycorrhizal plants. It is concluded that mycorrhizal plants exhibit increased capacity for phytoremediation of HM-contaminated soil, together with improved growth, and that their greater stress tolerance may derive from the protective role of PAs, and the transcriptional regulation of stress-related genes.