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USE OF POPLAR FOR REHABILITATION OF METAL POLLUTED SOILS

Van Anh LE THI¹, Mathieu POTTIER¹, Annabelle DEJARDIN², Gilles PILATE² and Sébastien THOMINE¹

¹Institut des Sciences du Végétal, CNRS UPR 2355, 91198 Gif-sur-Yvette Cedex, France

²Unité Amélioration Génétique et Physiologie Forestières, UR0588, INRA-Val de Loire, 45075 Orléans Cedex 2, France

Corresponding author email: lethi@isv.cnrs-gif.fr

ABSTRACT

Phytoextraction is the use of plants to remove heavy metals from soils contaminated by industrial activities, such as smelting or disposal of high metal wastes. Poplar is well adapted for phytoextraction because it tolerates growth on contaminated soils and produces high biomass. In addition, poplar is also suitable for molecular genetic studies because its genome was sequenced in 2006 and it is amenable to transgenic technologies. However, previous reports indicated that metal accumulation was the highest in leaves causing metal return to the soil after leaf abscission. In order to circumvent this problem, genetic engineering can be used to direct metal accumulation in poplar trunks using a relevant metal transporter gene under the control of a wood-specific promoter.

We first focused our study on the functional characterization of suitable metal transporter genes. We selected candidate transporters potentially controlling metal storage in the vacuole, the main compartment where heavy metals are accumulated in plant cells. The NRAMP (Natural Resistance Associated Macrophage Protein) and the IREG (Iron Regulated Gene) metal transporter families, which are still poorly characterized in poplar, have been investigated. Based on the knowledge gained from their homologues in *Arabidopsis thaliana*, PtNRAMP3.1 and PtNRAMP3.2 are putatively involved in metal release from the vacuole while PtIREG1 is putatively involved in metal sequestration in the vacuole. We showed that both PtNRAMP3.1 and 3.2 were functional metal transporters but only PtNRAMP3.2 was targeted to the vacuolar membrane. In contrast, PtNRAMP3.1 was targeted to intracellular vesicles. In addition, the function of PtIREG1 putative metal transporter gene was investigated by heterologous expression in yeast: PtIREG1 was shown to be able to complement the hypersensitivity of mutant strains to Ni but not to other metals, including Co, Cd, Fe, Mn and Zn.

Transgenic poplars overexpressing PtNRAMP3.1 or PtNRAMP3.2 were assessed for metal tolerance and accumulation. When grown on non-contaminated soil, wild-type and transgenic poplar lines exhibited clear differences in leaf metal concentrations (Cu, Fe, Mg, Mn and Zn). Metal accumulation will also be measured in transgenic poplar lines grown on metal-contaminated soil. Arabidopsis and poplar plants overexpressing PtIREG1 as a fusion with the fluorescent protein GFP (PtIREG1-GFP) are being generated. This should allow identification of the subcellular compartment where PtIREG1 resides and analysis of the effect of PtIREG1 expression on metal tolerance and accumulation. Besides, PtIREG1-GFP under control of the CAD promoter, which targets expression in poplar xylem cells, was introduced in poplar to specifically drive metal accumulation in wood.

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