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Production of *ipt*-expressing white poplar lines (*Populus alba* L.) with abnormal root morphology

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Abstract — *Agrobacterium*-mediated transformation of white poplar explants was performed using the pMAT-MT construct derived from the *ipt*-type MAT22 plasmid carrying the $PsMT_{A1}$ cDNA encoding a pea metallothionein. *In vitro* shoot regeneration occurred on antibiotic-free medium and transformants were identified by visual selection. Beside the expected abnormal *ipt*-shooty phenotype, shoots with normal morphology were recovered. Interestingly, an additional phenotype characterised by a remarkable root development was also observed.

Key words: *ipt* gene, metallothionein, morphological marker, phytoremediation, *Populus alba*.

INTRODUCTION

Since the remediation of metal-contaminated soils requires expensive technologies, phytoremediation, a novel cost-effective strategy for the clean-up of polluted soil, has been developed (Glick 2003). Poplar is a model system for phytoremediation, due to its ability to withstand environmental stresses, the extensive root system and the high water uptake (Peuke and Rennenberg 2006). The white poplar (*Populus alba* L.) clone 'Villafranca', a strong biomass producer with optimal performances in short rotation cultures (Confalonieri *et al*. 2000), has been used for several biotechnological applications. In view of the possible use of this clone for phytoremediation, *Agrobacterium*-mediated transformation with the $PsMT_{A1}$ gene from *Pisum sativum*, inserted into the plasmid MAT-MT, was carried out. The MAT (Multi-Auto-Transformation) vector system (Ebinuma and Komamine 2001), used to transform white poplar, represents an innovative tool for the production of 'marker-free' GM plants. The $PsMT_{A1}$ gene encodes a metallothionein with attractive features for phytoremediation since it is able to bind Cu *in planta* and showes strong affinities for Zn and Cd (Evans *et al*. 1992).

MATERIAL AND METHODS

The pMAT-22 vector used in this study, supplied by Dr. Yamada (Nippon Papers Industries, Tokyo), carried the *ipt* gene encoding *A. tumefaciens* isopentenyl transferase under the control of its endogenous promoter. Expression of the *ipt* gene induces the abnormal *ipt*-shooty phenotype and allows the visual selection of transformants. The normal phenotype is then restored by removing the *ipt* sequence throughout the yeast site-specific recombination system R/RS (Araki *et al.* 1987).

The *ipt* oncogene from *A. tumefaciens* stimulates regeneration by the organogenic pathway (Zuo *et al.* 2002). Cytokinin overproduction, caused by the *ipt* gene expression, induces the abnormal *ipt*-shooty phenotype characterised by reduced apical dominance. Moreover, shoots do not root on a hormone-free medium and new abnormal shoots and calli are continuously produced at the contact sites with agar medium.

Transformation was carried out as described by Confalonieri *et al*. (2000).

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RESULTS AND DISCUSSION

The expected *ipt*-shooty phenotype was observed during the regeneration step. In addition, shoots with normal morphology were also recovered. The normal phenotype may represent untransformed shoots actively growing in non selective medium; however the same phenotype may also be due to early excision events occurring in transformed tissues, as a consequence of precocious activation of the yeast recombinase. This response is known as 'single-step transformation', since it allows to recover 'marker-free' transgenic lines without an *ipt*-shooty intermediate phenotype. PCR analyses performed on each single *ipt*-shooty line confirmed the presence of the $PsMT_{A1}$, *ipt*, *nptII* and *R* sequences located within pMAT-MT, while the same analyses performed on normal shoots allowed to discriminate between untransformed and 'marker-free' transgenic lines (not shown).

Beside the *ipt*-shooty and normal phenotypes, an additional abnormal phenotype was observed, characterized by extensively developed roots. In Fig. 1A and B, two different GM poplar shoots, expressing the *ipt* gene and carrying this abnormal phenotype are shown. The root length was significanly higher than in wild type plants and the phenotype differed remakably from the typical *rol* ('hairy root') phenotype caused by the expression of the *rolABC* genes from *Agrobacterium rhizogenes* (Fig. 1C). As previously reported, the overexpression of *ipt* gene is responsible for reduced and/or absent rooting, a feature which is completely in contrast to the occurrence of the 'highly-rooting' phenotype. However, this morphology was recently described by LUO *et al.* (2005) in transgenic tomato expressing the *ipt* gene and also in GM cotton and cassava engineered with the same gene (TAYLOR *et al.* 2004). It has been reported that the auxin-producing shoot tips and the cytokinin-producing root tips reciprocally interact and influence the level of the active auxin/cytokinin pool (ALONI *et al.* 2005). The availability of poplar *ipt* GM lines with abnormal root development will possibly help understanding the relationship between auxin and cytokinin within these tissues.

The reported data not only support for the choice of *ipt* gene as a reliable morphological

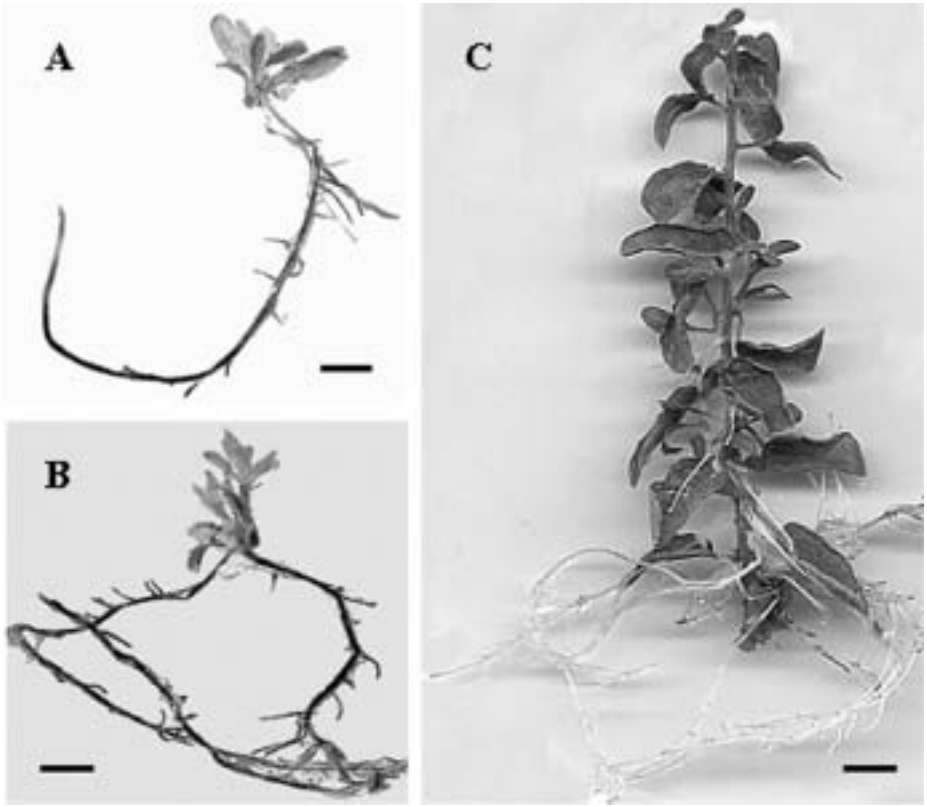

Fig. 1 — A and B, transgenic *ipt* white poplar lines showing the abnormal 'highly rooting' phenotype. C, transgenic *rol* white poplar line showing the 'hairy root' phenotype. Bar = 1 cm

marker but also offer novel interesting material for further studies on phytohormone metabolism.

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