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Evaluation of the expression level of the endogenous marker *poUBI* gene for studies on transgene stability in *bar* and *StSy* GM poplars

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Abstract — This work reports on the isolation and molecular characterization of the *poUBI* cDNA encoding polyubiquitin from white poplar (*Populus alba* L. cv 'Villafranca'). Expression analysis was performed on different poplar organs and tissues, at different developmental stages and in relation to the growth/dormancy cycle. Information concerning the steady-state level of *poUBI* transcripts *in planta* are required to better evaluate the possible use of this gene as endogenous marker for studies on long-term transgene stability in genetically modified white poplars.

Key words: genetically modified tree, polyubiquitin, transcript stability, white poplar.

INTRODUCTION

The genus *Populus* is a model system in forest tree biotechnology (Confalonieri *et al.* 2003). Long-term field trials are required to test GM trees and assess their potential environmental implications.

It has been reported that the activity of the constitutive 35SCaMV promoter, used to direct transgene expression in GM plants, is dependent on organ type/position and season (Hawkins *et al.* 2003). When transgene expression studies are performed over a long-time period, the parallel investigation of endogenous marker genes is strongly recommended to better assess the physiological state of both GM and untransformed plants. The expression patterns of *poUbi* genes encoding multiple copies of the protein ubiquitin have been evaluated in annual plants, due to their possible use as internal controls in quantitative expression analyses. Ubiquitin covalently binds a variety of nuclear, cytoplasmic and membrane proteins through complex reactions which finally lead to protein degradation (Varshavsky 1997). The ubiquitin-proteasome pathway represents the main mechanism for protein degradation in eukaryotes, responsible for the regulation of cell cycle, differentiation, programmed cell death and plant-microbe interactions (Singh *et al.* 2002; Schlogelhofer *et al.* 2006; Zeng *et al.* 2006).

MATERIAL AND METHODS

The 447 bp *poUBI* cDNA was amplified from poplar genomic DNA using degenerated oligonucleotide primers. The cDNA was purified from agarose gel and subsequently cloned into the pTZ57R vector (InsT/A clone™ PCR Product Cloning kit, M-Medical S.r.l.). DNA sequence similarity searches using the basic BLAST algorithm were performed at the website http://www.ncbi.nlm.nih.gov/. The *poUbi* cDNA (Accession no DQ056362) contains an open reading frame (ORF) coding for the ubiquitin monomer (76 amino acids). The 5'-end of the cDNA contains a truncated ORF encoding a partial ubiquitin monomer spanning only 33 amino acids, while another truncated ORF coding for 39 amino acids is located at the 3'-end. The *poUbi* expression pattern was investigated in white poplar tissues by northern blot hybridization and densitometric analysis of hybridization bands was

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carried out using a Biostep GmbH apparatus with the argus X1 3.3.0 software.

RESULTS AND DISCUSSION

In the present work, the isolation and molecular characterization of the *poUBI* cDNA encoding polyubiquitin from *Populus alba* L. cv 'Villafranca' is described. The availability of a molecular probe for endogenous *poUbi* genes will allow detailed expression analyses of GM white poplars engineered with the *bar* gene for herbicide tolerance and with the *StSy* gene for resveratrol production, respectively (Confalonieri *et al.* 2000; Giorcelli *et al.* 2004). The *poUbi* steady-state level was remarkably high in young leaves collected in spring from two-year old plants, soon after bud flush (Fig. 1, y). A decreased amount of *poUbi* mRNA was observed in mature leaves (Fig. 1, m), harvested in summer during the full vegetative growth, while the expression in dormant tissues was strongly reduced (Fig. 1, d). A 3-fold enrichment of *poUbi* transcript was evident in senescent leaves (Fig. 1, s), compared to dormant tissues. According to the current literature (Bhalerao *et al.* 2003), the *poUbi* mRNA is among the most abundant transcripts in poplar young leaves, but *poUbi* transcripts are also frequently found in autumn leaves where an enrichment factor of approximately 3, compared to young leaves, is observed (Bhalerao *et al.* 2003). Winter buds (Fig. 1, wb) revealed a very low transcript abundance, thus confirming down-regulated expression of *poUbi* genes in quiescent tissues during the cold season.

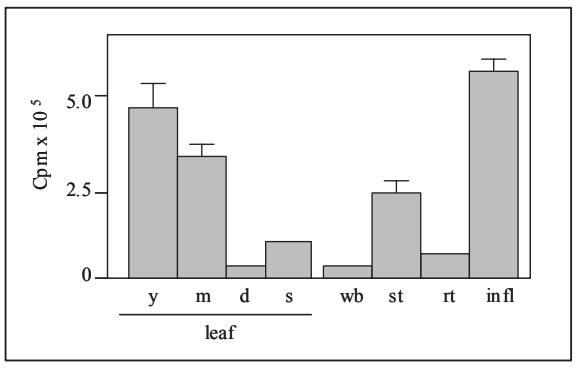

Fig. 1 — Evaluation of the steady-state levels of *poUbi* mRNA in *Populus alba* cv 'Villafranca'. Northern blot analysis was performed on young (y), mature (m), dormant (d) and senescent (s) leaf tissues. In addition, winter buds (wb), stems (st), roots (rt) and female inflorescences (infl) were examined. Cpm, counts per minute.

The steady-state level of *poUbi* transcript was also measured in stems and roots (Fig. 1, st and rt) collected in summer from two-year old plants. Finally, a very high expression level was found in the female inflorescences (Fig. 1, infl). The up-regulation of *poUbi* genes in plant reproductive tissues has been reported (Singh *et al.* 2002). The resulting *poUbi* expression profiles obtained *in planta* will represent a useful reference in future researches, carried out with GM poplars, to assess the level of transgene stability and other possible unexpected physiological responses associated to long-term tree cultivation. The availability of endogenous marker genes will accelerate the evaluation of GM poplars for commercial purposes.

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