

SHORT- AND LONG-TERM COLD-INDUCED GENE EXPRESSION IN WHITE POPLAR

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Low temperatures determine a “physiological dehydration”, because of a reduction of water transport at the root level. Cold stress affects plants determining changes at developmental, morphological, physiological and biochemical levels. All these changes involve precise changes in gene activity and synthesis of specific proteins that, possibly through cold acclimation, allow plant survival. To date, many stress-inducible genes have been isolated, especially in herbaceous plants, and in trees also. However comparative analyses between genotype of one and the same species, to analyze genetic variability in gene regulation patterns, are rare.

Populus is a genus of great economic value in Italy; from a scientific point of view, poplar is a very favorable model plant for genomic studies in trees: the nucleotide sequence of the entire genome of black cottonwood (*Populus trichocarpa*) has been determined; *Populus* genome is relatively compact (~500 Mbp) compared with other tree species; beside genome sequencing, an EST collection from poplar, aspen, cottonwood and their hybrids has already grown to >150,000.

With the final scope of studying genetic variability in gene regulation patterns, a set of genes induced by low temperature was isolated in the clone “Villafranca” of *Populus alba* and characterized. White poplar plants in the stage of leaf burst were maintained for two weeks at 25°C, then transferred to 4°C for 6, 12, 24, 48 hours, and mRNAs were isolated from leaves. Two differential cDNA libraries were constructed using the method of “Suppression Subtractive Hybridisation” (SSH) (Diatchenko et al., 1996), after short- (6 hours) or long- (48 hours) cold treatments.

Two-hundred-sixty isolated clones were sequenced and compared to databases. Fifty-four sequences resulted specifically induced after 6 h of cold treatment, 33 at both 6 and 48 h, and 82 specifically induced at 48 h of cold treatment. The putative products of isolated genes can be classified into different groups: i) proteins having a direct role in stress protection, as an early-responsive dehydration protein and a mannose/glucose lectin; ii) involved in signal transduction, as serine/threonine kinases/phosphatases and a calcium-calmodulin dependent kinase; iii) regulating gene expression, as MYB transcription factors, zinc-finger proteins; iv) involved in cell cycle activity regulation, as a phragmoplast-associated kinesin and other proteins. For each gene, the corresponding code in Arabidopsis was determined and an analysis using the Arabidopsis Mapman software allowed to determine the involvement of such genes in their metabolic pathway. For 33 cold-induced isolated genes the function is still unknown.

Analysis of upstream regions of isolated genes in the *P. trichocarpa* genome evidenced the occurrence of conserved cis elements involved in stress response regulation of gene activity. ARR1-binding elements – related to cytokinin-induced transcription factors – are largely represented.

Other elements typically related to defense responses – MYB, MYC, WRKY – were found. By contrast, DRE and ABRE elements were rare.

We have also analysed the expression of isolated genes at 6, 12, 24, 48 h of cold treatment and after a 24 h of recovery and established expression patterns to be used in comparative analyses in other white poplar genotypes from different environments.