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Poster Abstract - E.22

ISOLATION AND CHARACTERIZATION OF COLD-INDUCED GENES IN WHITE POPLAR

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Poplar is a very favorable model plant to be studied to understand molecular processes of growth, development and responses to environmental stimuli in trees: the nucleotide sequence of the entire genome of black cottonwood (*Populus trichocarpa*) has been recently 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.

Among abiotic stresses, we are especially interested with dehydration. For example, 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. Generally, both biotic and abiotic stresses induce common response mechanisms, including the biosynthesis of heat shock proteins, of pathogenesis-related proteins, of metallothioneins, the activation of oxidative stress response, osmoregulation mechanisms, etc., suggesting the occurrence of common gene regulation mechanisms: to date, many stress-inducible genes have been isolated, especially in herbaceous plants, and in trees also.

To isolate genes activated by abiotic stresses and contribute to clarify mechanisms of tolerance, a transcriptomic approach by the construction of differential cDNA libraries is needed. During last years, we have isolated and partially characterised genes differentially expressed in white poplar (*Populus alba*) leaves from plants subjected to short- (6 hours) or long- (48 hours) cold treatments. Two differential cDNA libraries were constructed using the method of "Suppression Subtractive Hybridisation" (SSH) (Diatchenko et al., 1996). Two-hundred-sixty isolated clones were sequenced and compared to databases.

Freezing tolerance is a multigenic trait and the first data from our experiments show that many genes are activated by cold treatments. 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 many 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 motifs presumably involved in early stress response regulation of gene activity.

We are now verifying the differential expression of many cDNAs by Northern blot hybridisations and semi-quantitative RT-PCR.