CHARACTERIZATION OF COLD INDUCED GENES FROM CYPRESS

PEDRON L.*, BALDI P.**, HIETALA M.A.***, LA PORTA N.*

*) IASMA Research Centre, Natural Resources Department, Via E. Mach 2, 38010 San Michele all’Adige (Italy)
**) IASMA Research Centre, Genetics and Molecular Biology Department, Via E. Mach 2, 38010 San Michele all’Adige (Italy)
***) Norwegian Forest Research Institute, Høgskoleveien 8, 1432 Ås (Norway)

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Cold is one of the most important and studied abiotic stresses. In fact, about two thirds of the world’s perennial landmass is subjected to winter temperatures below the freezing point, and about half of it must cope with temperatures below -20°C. It is therefore not surprising that many attempts have been made to improve cold resistance in plants, either by classical breeding or by gene transfer. This molecular study on cypress (Cupressus sempervirens) is focused on the isolation and cloning of sequences differentially expressed during the exposure of the plant to low temperatures. For this purpose a subtractive approach has been used, based on the method “PCR-Select”, using the Clontech kit. Two different genotypes of cypress were grown under controlled growth-chamber conditions for 21 days at 22°C and then the temperature were lowered at 2°C for 15 days. Samples of leaf were extracted before the treatment (control) and after 1, 2, 3, 7 and 15 days from the beginning. Samples of mRNA were extracted and used as starting material to obtain cold-regulated genes. With this method a large number of cDNA fragments were obtained, cloned into a plasmid vector and sequenced. cDNA sequences were analysed and compared with DNA and protein databases using the BLAST server at NCBI. A total of 108 gene were analyzed, obtaining 90 unique sequences. Of these 59 resulted to have good homology with known sequences. The greater part of these showed high homology with genes that in other plant species have been found to be regulated by cold or oxidative stress.

A Real-Time PCR experiment was performed on 24 sequences in order to confirm that in cypress these genes are cold regulated. The results showed a clear induction of different genes and in some cases a distinction between the two genotypes used. Even if the trend is always similar, the two different genotypes showed a quantitatively different expression.