Isolation of Cold Responsive Genes in Carrot by Suppression Subtractive Hybridization

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Low temperature is one of the major abiotic factors which limit growth, productivity and geographical distribution of plants. Some plants have adapted to survive at very low or even freezing temperatures and, hence, occupy high altitude areas such as mountains and Arctic or Antarctic regions. The mechanisms that a plant uses to survive at such low temperatures have been the subject of intense research for the past few years and modern molecular tools have helped provide understanding of some aspects of low temperature tolerance in plants. Suppression subtraction hybridization (SSH) is a PCR based method used to selectively amplify differentially expressed cDNAs and simultaneously suppress the non-target cDNA. *Daucus carota* is cultivated widely in tropical and temperate regions, but this species does best in cool climates. The aim of this project is to identify potential cold responsive genes which are upregulated during cold stress in carrot. To achieve this, a SSH library was constructed from RNA isolated from the leaves of control plants and cold stressed carrot plants. Out of the hundreds of clones obtained from the forward SSH library, some were sequenced, from which 52 sequences of promising clones were submitted to the EST NCBI database. Sequence analysis revealed that the functions of the identified genes were diverse and have significant roles in signal transduction, osmolyte synthesis and transport, photosynthesis, transcription factors, protein folding, nucleic acid synthesis, transcription and translation. Dot blot analysis revealed the relative expressions of various genes during cold stress. Future studies on this aspect will help increase our understanding of the complex mechanisms of abiotic stress response, in particular cold stress, and may help further the development of cold tolerant transgenic crops.