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Poster Abstract - B.12

CHARACTERIZATION OF A *KNOTTED1*-LIKE GENE OF *HELIANTHUS TUBEROSUS*

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Several gene families cooperate in meristem signalling; among these *Knotted*-like *Homeobox* (*KNOX*) genes have been shown to play a crucial role. The homeodomain of *KNOX* genes contains a conserved secondary structure described as "helix-loop-helix-turn-helix" and conserved regions at the N-terminal: the amphipathic helix and the ELK domain. *KNOX* genes, belonging to the TALE (Three Amino acid Loop Extension) superclass homeobox genes, are further divided into two classes by amino acid sequence differences in the third helix of the homeodomain and distinctive expression patterns. The combined effects of class 1 *KNOX* genes are required for the prevention of improper stem cell differentiation in the peripheral zone of the SAM. The present work is aimed to characterize the expression pattern of a class 1 *KNOX* like gene isolated from *Helianthus tuberosus* (2n = 6x = 10 2) and its involvement on morphogenetic competence expressed by two somaclones (EMB-2 and EMB-9) isolated from the interspecific tetraploid (2n = 4x = 68) hybrid *H. annuus* x *H. tuberosus*. The reconstructed full-length cDNA sequence (*HtKNOT1*, 1398 bp), obtained from 3' and 5' RACE, contained a 1089 bp CDS, 54-nucleotides of 5'-untranslated region (UTR), and 255-nucleotides of 3'-UTR. The predicted protein (HtKNOT1) displayed 362 amino acids with a calculated molecular mass of 40.2 kDa. A BLAST search against the protein database of the National Center for Biotechnology Information (NCBI) indicated that the encoded protein shared high sequence identity with members of the class I *KNOX* subfamily. Sequence information from the *HtKNOT1* cDNA was used to design specific primers and to isolate the full-length exons/introns region of the gene. The expression pattern of *HtKNOT1* was examined in plants of *H. tuberosus* by both RT-PCR and *in situ* hybridisation. *HtKNOT1* has been observed to be highly expressed in vegetative shoots and stems. Weak presence of transcripts was detected also in incipient leaf primordia and young leaves. In the last years, it has been demonstrated that the ectopic reactivation of *KNOX* genes may be sufficient to restore the meristematic potential in differentiated cells. Here, we report that misexpression of *HtKNOT1* is intimately linked to the development of ectopic shoots and somatic embryos on leaves of the somaclone EMB-2. Misexpression of *HtKNOT1* was also observed in somatic embryos developed from intact adventitious roots of *in vitro*-grown plantlets of the variant clone EMB-9. The effect of exogenous hormonal treatments on *HtKNOT1* expression was evaluated. Notably, massive shoot/embryo regeneration along with a strong accumulation of *HtKNOT1* transcripts was induced in EMB-2 non-epiphyllous leaves by *in vitro* zeatin treatment. However, epiphyllous structures were not formed in zeatin-treated control leaves, despite an enhancement of *HtKNOT1* expression, suggesting that *HtKNOT1* is not the unique factor, to trigger ectopic morphogenesis in EMB-2 leaves.

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