

TOMATO PROSYSTEMIN: STRUCTURAL AND FUNCTIONAL CHARACTERIZATION

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Systemin (Sys) is a 18-amino-acid peptide hormone which, upon insect attack, is released from its precursor protein, Prosystemin (Prosys) to initiate a complex signaling cascade that leads to the production of defense compounds (1). The tomato genome contains only one copy of the *Prosys* gene; it is composed of 4176 bp and is structured into 11 exons, of which the last one codes for Sys. Sys is considered one of the key component of the tomato plant molecular defense against insect herbivores. Despite that, it was recently demonstrated that the precursor, deprived of the Sys aminoacidic sequence, promotes defense responses (2). This observation prompts us to investigate the biochemical and structural features of the Prosys protein. To this purpose *Prosys* cDNA was amplified (using site-specific primers), cloned into pETM11 vector (useful to link His-tag at N-terminal region of these proteins) and expressed in BL21 (DE3) *E. coli* strain. The recombinant protein was purified by three chromatographic steps: Immobilized Metal Affinity Chromatography (IMAC), Ion Exchange Chromatography (IEC) and Size Exclusion Chromatography (SEC). After each step of purification, protein purity was assessed on 15% SDS-PAGE. Since the beginning Prosys showed peculiar behaviours (3) as observed by SDS-PAGE and chromatographic tools, further analyses have been performed. Bioinformatic tools allowed us to evaluate the intrinsic features of the protein and structure prediction servers have been used to analyze the secondary and tertiary structure of the prohormone. Finally, functional experiments in plants have been carried out to evaluate its biological activity.