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# Sequence Analysis and Molecular Cloning of PROTEOLYSIS 6 in Tomato

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**Abstract :** The evolutionarily conserved N-end rule pathway marks proteins for degradation by the Ubiquitin Proteosome System (UPS) based on the nature of their N-terminal residue. Proteins with a destabilizing N-terminal residue undergo a series of condition-dependent N-terminal modifications, resulting in their ubiquitination and degradation. Intensive research has been carried out in Arabidopsis previously. The group VII Ethylene Response Factor (ERFs) transcription factors are the first N-end rule pathway substrates found in Arabidopsis and their role in regulating oxygen sensing. ERFs also function as central hubs for the perception of gaseous signals in plants and control different plant developmental including germination, stomatal aperture, hypocotyl elongation and stress responses. However, nothing is known about the role of this pathway during fruit development and ripening aspect. The plant model system Arabidopsis cannot represent fleshy fruit model system therefore tomato is the best model plant to study. PROTEOLYSIS6 (PRT6) is an E3 ubiquitin ligase of the N-end rule pathway. Two homologs of PRT6 sequences have been identified in tomato genome database using the PRT6 protein sequence from model plant Arabidopsis thaliana. Homology search against Ensemble Plant database (tomato) showed Solyc09g010830.2 is the best hit with highest score of 1143, e-value of 0.0 and 61.3% identity compare to the second hit Solyc10g084760.1. Further homology search was done using NCBI Blast database to validate the data. The result showed best gene hit was XP_010325853.1 of uncharacterized protein LOC101255129 (Solanum lycopersicum) with highest score of 1601, e-value 0.0 and 48% identity. Both Solyc09g010830.2 and uncharacterized protein LOC101255129 were genes located at chromosome 9. Further validation was carried out using BLASTP program between these two sequences (Solyc09g010830.2 and uncharacterized protein LOC101255129) to investigate whether they were the same proteins represent PRT6 in tomato. Results showed that both proteins have 100 % identity, indicates that they were the same gene represents PRT6 in tomato. In addition, we used two different RNAi constructs that were driven under 35S and Polygalacturonase (PG) promoters to study the function of PRT6 during tomato developmental stages and ripening processes.

**Keywords :** ERFs, PRT6, tomato, ubiquitin
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