

# **Molecular cloning and characterization of a granulin-containing cysteine protease SPCP3 from sweet potato (*Ipomoea batatas*) senescent leaves.**

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## **Abstract**

Granulins are a family of evolutionarily ancient proteins that are involved in regulating cell growth and division in animals. In this report a full-length cDNA, SPCP3, was isolated from senescent leaves of sweet potato (*Ipomoea batatas*). SPCP3 contains 1389 nucleotides (462 amino acids) in its open reading frame, and exhibits high amino acid sequence homologies (ca. 64-73.6%) with several plant granulin-containing cysteine proteases, including potato, tomato, soybean, kidney bean, pea, maize, rice, cabbage, and *Arabidopsis*. Gene structural analysis shows that SPCP3 encodes a putative precursor protein. Via cleavage of the N-terminal propeptide, it generates a protein with 324 amino acids (from the 139th to the 462nd amino acid residues), which contains two main domains: the conserved catalytic domain with the putative catalytic residues (the 163rd Cys, 299th His and 319th Asn) and the C-terminal granulin domain (from the 375th to the 462nd amino acid residues). Semi-quantitative RT-PCR and protein gel blot hybridization showed that SPCP3 gene expression was enhanced significantly in natural senescent leaves and in dark- and ethephon-induced senescent leaves, but was almost undetectable in mature green leaves, veins, and roots. Phylogenetic analysis showed that SPCP3 displayed close association with a group of plant granulin-containing cysteine proteases which have been implied to be involved in programmed cell death. In conclusion, sweet potato SPCP3 is a functional, senescence-associated gene. Its mRNA and protein levels were significantly enhanced in natural and induced senescing leaves. The physiological role and/or function of SPCP3 associated with programmed cell death during leaf senescence were also discussed.