Title Cloning and gene expression analysis of phospholipase C in wounded spinach leaves during postharvest storage

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Abstract

Membrane destabilization in plant cells can be caused by mechanical injuries, pathogen attacks, environment stresses or senescence. Wounds represent vulnerable points that may lead to severe damage and can compromise organ survival rates. Loss of membrane integrity is often associated with lipid peroxidation or degradation of phospholipids. This work focuses on the isolation of phospholipase C (PLC) in spinach leaves and the investigation of its role in membrane destabilization during leaf senescence and after wounding. Degenerate primers were used to amplify a 270 bp fragment with RT-PCR and a full length *SoPLC* mRNA was isolated using a RACE approach. The mRNA was 2290 bp in length and contained an open reading frame of 1765 bp. The predicted amino acid sequence showed high similarity with PLC2 and PLC7 of *Arabidopsis*. Gene expression analyses showed that *SoPLC* was down-regulated by wounds and up-regulated by detached induced senescence. Membrane integrity was evaluated by lipid peroxidation and HPLC phospholipid analyses. Phospholipase C (PLC) and D (PLD) enzyme activities were determined in detached leaves incubated in the dark at 4 or 20 °C. Lipid peroxidation and enzymatic activities were mainly affected by senescence and temperature rather than wounds. Lipid peroxidation did not change at 4 °C with TBARS values ranging from 3 to 4 nmol g^{-1} FW. Leaves incubated at 20 °C showed an increase of TBARS from 4 to 12 nmol g^{-1} FW. PLC and PLD enzymatic activities in leaves incubated at 20 °C significantly increased after seven days with higher values in wounded leaves (3300 pKat mg^{-1} prot) compared with control (550 pKat mg^{-1} prot.).