Title Chlorophyllase versus pheophytinase as candidates for chlorophyll dephytilation during

senescence of broccoli

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Citation Journal of Plant Physiology, Volume 168, Issue 4, 1 March 2011, Pages 337-343

Keywords Chlorophyll breakdown; Chlorophyllase; Hormone treatments; Pheophytinase;

Senescence

Abstract

Degradation of chlorophylls during senescence is a highly regulated process which requires the concerted action of several enzymes. Traditionally, it has been stated that the dismantling process of the chlorophyll molecule begins with a dephytilation step, followed by Mg2+ removal and other breakdown reactions. Recently, new evidence suggests the possibility of a rearrangement in the first two steps of this process, occurring Mg²⁺ removal prior to the loss of the phytol side chain. With the purpose of approximating to the real sequential order of these reactions and to assess if dephytilation occurs on intact (catalyzed by chlorophyllase) or Mg-free (catalyzed by pheophytinase) chlorophyll, expression of both genes was analyzed in broccoli tissue during senescence. Samples of broccoli florets treated with plant hormones, such as cytokinin and ethylene were utilized, as to assess the effect of such compounds on the expression of these genes. Results showed that chlorophyllase expression did not correlate to typical expression patterns for genes related to senescence, since a decrease in expression during senescence was found for one of the two chlorophyllase genes analyzed, and the hormonal-treatment effects on gene expression did not match those observed on chlorophyll content for both chlorophyllase genes. Pheophytinase expression patterns, on the other hand, displayed an increase in the first 3 days of induced senescence, followed by lower expression values towards the end of the experiment. Samples subjected to postharvest treatments mostly showed an inhibition of pheophytinase expression, especially in samples in which degradation of chlorophylls had been delayed. These results suggest that pheophytinase expression correlates to the visual manifestation of postharvest treatments, supporting the possibility that this enzyme is responsible for the dephytilation step in chlorophyll breakdown.