Title Transgenic broccoli (*Brassica oleracea* var. *italica*) with antisense chlorophyllase (BoCLH1) delays postharvest yellowing

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Abstract

Postharvest yellowing in broccoli is known to result from chlorophyll degradation, with chlorophyllase the first enzyme to degrade chlorophyll. In broccoli, three putative chlorophyllase genes (*BoCLH1*, *BoCLH2*, and *BoCLH3*) were cloned using degenerate primers from the conserved regions of known chlorophyllases. Among these three genes, only *BoCLH1* is transcribed during the course of broccoli postharvest senescence. A chimeric construct with the antisense *BoCLH1*, driven by the CaMV 35S promoter and Nos-terminator and harboring the hygromycin resistance gene, was used for *Agrobacterium*-mediated transformation to study the effects of the antisense *BoCLH1* gene on the postharvest senescence of broccoli. From a total of about 90 primary transformants, 35 individuals were selected and grown for further studies, with 22 of these grown to maturity. Based on the Chl retention rate on Days 4 and 5, respectively, 45% of the detached florets and over 60% of the detached leaves of the selected transformants exhibited slower yellowing when stored at 20 °C in darkness. Southern blot analyses were conducted to eliminate the possible non-independent transformants and investigate the insertion patterns and copy numbers. Only a few lines with simple insertion site and copy number and postharvest yellowing retardation effects were self-pollintated for further evaluation. Northern analyses showed antisense *BoCLH1* mRNA transcripts on the day of harvest, the levels of which gradually decreased 4–5 days postharvest when stored at 20 °C in darkness. Positive correlations between the antisense *BoCLH1* transcripts and slower postharvest yellowing were noted in some selected lines. Only 1–2 days delay in yellowing was observed in the selected antisense *BoCLH1*-positive transformants. No individual antisense *BoCLH2* or *BoCLH3* transformants showed significant slowing of postharvest yellowing. The results suggested genes other than the *BoCLH*-Chlases obtained in the present study might also be essential in the yellowing process.