Title Suppression of the cysteine protease, aleurain, delays floret senescence in *Brassica*

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Abstract

An aleurain-like protein, BoCP5, is up-regulated during harvest-induced senescence in broccoli floret and leaf tissue. BoCP5 is most closely related to an *Arabidopsis* protein (91%, AAF43041) and has 71% identity to barley aleurain (P05167). The mRNA for this gene accumulates within 6 h after harvest in broccoli florets, and its expression is reduced in tissue that has been held in senescence-delaying treatments (e.g. water, sucrose feeding, controlled atmosphere). The gene is also expressed in leaves during aging-related and harvest-induced senescence. Analysis of protein bands that cross-react with antibodies raised to the bacterial BoCP5 fusion protein, revealed prominent immunoreactive bands at ca. 26, 28, 31, and 38 kD in floret tissue. The 31 kD band was absent in protein extracts from leaf tissue. *Agrobacterium*-mediated transformation was used to produce transgenic broccoli plants with downregulated BoCP5. A reduction in the postharvest expression of BoCP5 in floret tissue was achieved for four transgenic lines in the current study. In three of these lines postharvest floret senescence (yellowing) was delayed, and florets contained significantly greater chlorophyll levels during postharvest storage at 20 °C than wild-type plants. Line 4 showed the greatest down-regulation of BoCP5, and in this line postharvest protease activity remained at pre-harvest levels, and the yield of soluble proteins extracted from florets after harvest was significantly greater than that of wild-type tissue.