Title Proteomic analysis of stress-related proteins in transgenic broccoli harboring a gene for cytokinin production during postharvest senescence

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Abstract

Our previous study revealed a cytokinin-related retardation of post-harvest floret yellowing in transgenic broccoli (*Brassica oleracea* var. *italica*) that harbored the bacterial isopentenyltransferase (*ipt*) gene. We aimed to investigate the underlining mechanism of this delayed post-harvest senescence. We used 2D electrophoresis and liquid chromatography–electrospray ionization–mass spectrometry/mass spectrometry for a proteomics analysis of heads of *ipt*-transgenic and non-transgenic inbred lines of broccoli at harvest and after four days post-harvest storage. At harvest, we found an accumulation of stress-responsive proteins involved in maintenance of protein folding (putative protein disulfide isomerase, peptidyl-prolyl cis–trans isomerase and chaperonins), scavenging of reactive oxygen species (Mn superoxide dismutase), and stress protection [myrosinase-binding protein, jasmonate inducible protein, dynamin-like protein, NADH dehydrogenase (ubiquinone) Fe-S protein 1 and stress-inducible tetratricopeptide repeat-containing protein]. After four days' post-harvest storage of non-transgenic broccoli florets, the levels of proteins involved in protein folding and carbon fixation were decreased, which indicates cellular degradation and a change in metabolism toward senescence. In addition, staining for antioxidant enzyme activity of non-transgenic plants after post-harvest storage revealed a marked decrease in activity of Fe-superoxide dismutase and ascorbate peroxidase. Thus, the accumulation of stress-responsive proteins and antioxidant enzyme activity in *ipt*-transgenic broccoli are most likely associated with retardation of post-harvest senescence.