Title The Production of Marker-Free Genetically Engineered Broccoli with Sense and Antisense ACC synthase 1 and ACC oxidases 1 and 2 to Extend Shelf-Life

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Abstract

The production of transgenic broccoli (*Brassica oleracea*) with increased shelf-life using an *Agrobacterium rhizogenes*-mediated co-transformation protocol is reported. An *Agrobacterium rhizogenes* Ri vector, pRi1855:GFP was constructed to allow expression of the green fluorescent protein to identify insertion of Ri T_L-DNA into plant cells. The *Brassica oleracea ACC synthase 1* and *ACC oxidase 1* and *2* cDNAs in sense and antisense orientations were co-transformed into GDDH33, a doubled haploid calabrese-broccoli cultivar. Transformation efficiency was 3.26%, producing 150 transgenic root lines, of which 18 were regenerated into mature plants. The floral buds from T_0 broccoli heads were assayed for post-harvest production of ethylene and chlorophyll levels. Buds from T_0 lines transformed with *ACC oxidase 1* and *2* constructs produced significantly less post-harvest ethylene at 20 °C than the untransformed plants and chlorophyll loss was significantly reduced over a 96 h post-harvest period. The T_0 plants transformed with sense and antisense *ACC synthase 1* had a significantly reduced 24 h post-harvest ethylene peak and delayed chlorophyll loss. A positive correlation between post-harvest bud ethylene production and chlorophyll loss was described by a regression. This demonstrates that the shelf-life of a very perishable vegetable may be increased up to 2 days at 20 °C by reducing post-harvest ethylene production.