

Title Ethylene insensitive and post-harvest yellowing retardation in mutant ethylene response sensor (boers) gene transformed broccoli (*Brassica oleracea* var. *italica*)

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Abstract

A mutant broccoli *ers* (ethylene-response-sensor, *boers*) gene was obtained through site directed mutagenesis by replacing the isoleucine with phenylalanine at the 62th residue. Two plasmids were constructed with this mutant gene regulated by the CaMV 35S promoter together with the *nptII* (kanamycin resistance gene) coding sequence and *hpt* (hygromycin resistance gene), respectively, for the pBI-mERSI62F and pSM1H-mERSI62F plasmids. Genetic transformation of the above two constructs *via A. tumefaciens* has been conducted to evaluate their effects on floret yellowing of harvested broccoli. Over a hundred transformants have been obtained on the selected cotyledon and hypocotyl explants. PCR and Southern analysis demonstrated integration of the transgenes in the transformants. However, through Southern hybridization, we determined that multi-site integration and DNA rearrangements had occurred in most transformants. Morphological and characteristic alternation such as slower plant growth, shorter plant height, easy branching, late bolting, and relative higher mortality in comparison with other transgenes were noted in some transformants. Transgenic lines showing delayed senescence in leaves and floral heads were obtained. The expression of transgene was confirmed by Northern blot analysis. The transformed progenies also showed ethylene insensitivity in seed germination, detached leaves and harvested florets. Nevertheless, in most lines, the yellowing was only delayed 1–2 days.