Shoot tip regeneration and optimization of Agrobacterium tumefaciens-mediated transformation of broccoli (Brassica oleraceae var. italica) cv. Green Marvel

ABSTRACT

A protocol of plant regeneration from shoot tips and optimization of Agrobacterium tumefaciens-mediated transformation of broccoli (Brassica oleracea var. italica) cv. Green Marvel have been developed. Shoot tip response was assessed on Murashige and Skoog (MS) medium supplemented with different concentrations of zeatin. The highest regeneration with a maximum of 13 shoots per explant was obtained on MS medium containing 1.5 mg l-1 zeatin. Primary selection of putative transformed explants was performed on the optimized regeneration medium (MS medium containing 1.5 mg l-1 zeatin and 80 mg l-1 kanamycin) for 60 days. The effects of preculture, acetosyringone and growth of bacterial culture were studied. Explants precultured on callus induction medium for 4 days prior to inoculation with A. tumefaciens with 200 lM acetosyringone resulted in improved transformation frequency. The Agrobacterium culture dilution of 1:5 and inoculation time of 30 min increased the efficiency of transformation of shoot tip explants. The results also indicated that 150 mg l-1 ampicillin alone was adequate to eradicate Agrobacterium growth in the SRM incorporated with the respective minimum inhibitory concentration of 80 mg l-1 kanamycin. The polymerase chain reaction (PCR) and Southern blot assays confirmed the transgenic status of the broccoli cv. Green Marvel regenerants. A transformation efficiency of 5 % was achieved based on the positive PCR results using the optimized procedure. The expression of luciferase reporter gene in the transformed cells and the transcription of AtHSP101 using RT-PCR further confirmed the transgenic status of the regenerated plants.

Keyword: Broccoli; AtHSP101; Luciferase gene; Southern blot; Agrobacterium tumefaciens