Stable Transformation of Suspension-Cultured *Glycyrrhiza inflata* Batalin Cells with *Agrobacterium tumefaciens*

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A protocol for the efficient genetic transformation of licorice (*Glycyrrhiza inflata* Batalin) cells in suspension culture using *Agrobacterium tumefaciens*-mediated T-DNA delivery is described. *G. inflata* cells in suspension culture were infected with *A. tumefaciens* strain LBA4404 harbouring the binary vector pCAMBIA1303, which contains the *b*-glucuronidase (GUS) reporter gene and a hygromycin resistance gene (*hpt* II), respectively, under the transcriptional control of the CaMV35S promoter. Optimal transformation efficiency was achieved with an *A. tumefaciens* suspension having an OD_{600} of 0.4 and a period of 24 h of co-cultivation with 3-day-old cells in a medium supplemented with 200 μM acetosyringone. The transgenic cell lines have been maintained in suspension subculture for 5 months. PCR and Southern blot analyses confirmed the stable integration of transgenes into the *G. inflata* genome. The introduced genes had no discernable effect on cell growth or accumulation of total licorice flavonoids in the transgenic cell lines. This study provides the basis for the development of transgenic *G. inflata* cells.

Key words: Agrobacterium tumefaciens, *Glycyrrhiza inflata*, Transformation, Flavonoids