D34 Expression of β-Glucuronidase(GUS) gene in Transgenic Flower Tobocco Plant

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형질전환된 꽃담배에서의 GUS 유전자의 발현

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Objective

Establishment of an effective transformation system as a basic step for gene manipulation in flower tobocco plant

Materials and Methods

Plant material : sterilized seeds of flower tobocco plant were germinated on MS medium without plant growth regulators at 25 ± 1℃ in the light.

Control medium : MS media supplemented with 0.1 - 0.5 mg/L 2,4-D or 0.1-0.5 mg/L NAA in combination with 1.0 mg/L BA

Selection medium : 0.1 mg/L NAA, 1.0 mg/L BA, 200 mg/L kanamycin and 300 mg/L cefotoxime.. 3 g/L phytagel and 30 g/L sucrose, pH 5.8

Culture condition: Culture at 25℃ in a 16 h light / 8 h dark photoperiod.

Agrobacterium vector and transformation :

Agrobacterium tumefaciens strain LBA 4404 containing a binary vector pBI 121 (carries the β-Glucuronidase(GUS) gene fuses to a CaMV 35s promotor). preculture : 2 days, coculture: 2 days, soaking time: 15 min

PCR analysis

PCR of Gus gene :Forward primer(5'-TGG ACA AGG CAC TAG CGG-3') and reverse primer(5'-ACC GCC AAC GCG CAA TAT GC-3').

PCR of *npt* II gene : Foward primer(5'GAG GCT ATC GGC TAT GAC TG-3') and reverse primer(5'-ATC GGG AGC GGC GAT ACC GTA-3')

Results and discussion

Putative transformed shoots were induced on MS selection media supplemented with 0.1 mg/L NAA, 1.0 mg/L BA, 200 mg/L kanamycin and 300 mg/L cefotoxime. After subculture on fresh selection medium these shoots were rooted on MS medium containing 100 mg/L kanamycin and 300 mg/L cefotaxime without plant growth regulators. PCR analysis indicated that the *npt*II and GUS gene were stably integrated into the nuclear genome of flower tobocco plant.

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Table 1. Effects of plant growth regulators to shoot formation after 60 days of culture from flower tobocco

Growth regulators(mg/L)			Callus induction(%)	Shoot formation(%)	No. of shoot/explant
2,4-D	NAA	BA			
0.1		1.0	-23.1		
0.5		1.0	31.2		
	0.1	1.0	72.1	55.2	4.2
	0.5	1.0	54.4	29.8	2.6

Table 2. Effects of kanamycin concentration on shoot formation after 60 days of culture from flower tobocco

Kanamycin conc.(mg/L)	Shoot formation(%)	No. of shoot / explant
0	48.7	3.9
50	12..8	1.5
100	7..5	0.4
200	0	0

[a]MS medium supplemented with 0.1 mg/L NAA and 1.0 mg/L BA was used

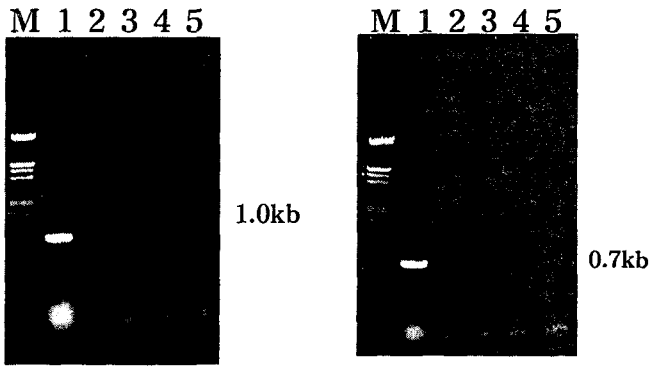

Figure 4. Agarose gel electrophoresis of PCR amplification products. M: size maker(λ /HindIII+ EcoR I); lane 1: positive control; lane 2: Negative control(amplified product from genomic DNA of non-trasformed plant); lane 3, 4 and 5: amplified product from genomic DNA of trasgenic plant. The arrows indicate approximately 1.0 kb of amplified GUS product(left) and 0.7 kb of amplified *npt* II product(right)