Improvement of Citrus Quality by Introduction of Sucrose Synthase and Fatty Acid Desaturase Genes

– The Conditions for Tissue Culture and DNA Introduction –

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ABSTRACT

We optimized the conditions for tissue culture of *Citrus sinensis* Osbeck 'Yoshida'. We also studied the effect of each parameter on the efficiency of DNA introduction into cells using the microprojectile bombardment DNA delivery system. The Murashige-Tucker(MT) medium was best for the growth of callus cells derived from the immature seeds. The optimum concentration of sucrose in the medium was 30 g/l for the growth of callus cells. Shoots were induced from the cells on the media supplemented with several combination of naphthaleneacetic acid(NAA) and thidiazuron. The highest shoot formation was obtained on a medium containing 10 mg/l of NAA and 0.1 mg/l of thidiazuron. The growth of the callus was stopped at the level of 100 mg/l of kanamycin in medium, which might be suitable for screening of transformants with nptII gene. For the introduction of DNA into cells by microprojectile bombardment, 900 psi of helium gas pressure, 1/4" of gap distance and 7.0 cm of target distance were optimum respectively.

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INTRODUCTION

For the sucessful transformation of a plant it is very important to establish the conditions for tissue culture. In this study we tried to optimize the condition for culture of callus cells induced from immature seeds of navel orange (*Citrus sinensis* Osbeck 'Yoshida') which is one of the important *Citrus* species in Cheju. Many factors are known to affect growth of callus cells. We studied the effects of the culture medium and concentration of sucrose in the medium on the growth of callus cells. Plant regeneration from cultured cells is usually regulated by plant growth regulators including auxins and cytokinins. We tested the effects of NAA as an auxin source and thidiazuron as a cytokinin source on shoot formation of the callus cells. The susceptibility of the cells to kanamycin was also tested to know the optimum concentration of the antibiotic in the medium for selecting transformants with kanamycin resistant gene, nptII. A foreign DNA can be introduced into plant cells by several ways. In this study we used the microprojectile bombardment DNA delivery system and dicussed the effects of several important parameters on the efficiency of DNA introduction into cells.

MATERIALS AND METHODS

The callus cells induced from the immature seed of navel orange (*Citrus sinensis* Osbeck 'Yoshida') were used as plant material. The cells were subcultured every 3 weeks and maintained on Murashige and Tucker(MT) medium.

The PDS-1000/He Biolistic Particle Delivery System (Bio-Rad) was used for introducing DNA into cells. The efficiency of DNA introduction was evaluated by comparing the relative scores of GUS gene expression in bombarded cells.

RESULTS AND DISCUSSION

Five types of tissue culture media, MT (Murashige and tucker, 1969), MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1981), GD (Gresshoff and Doy, 1974) and B5 (Gamborg et al., 1968) were tested to compare the relative growth of cells on each medium. Among the tested media, MT medium was best for cell growth (Table 1). The optimum concentration of sucrose in the medium was 30 g/l (Table 2).

Shoots were formed from the cells on media supplemented with several combination of NAA and thidiazuron via somatic embryogenesis (Fig. 1). Although shoot formation from the callus cells was observed even on the medium without growth regulators, the frequency was very low.

Table. 1. Effect of the culture medium on the growth of callus cells. The cells were cultured on each medium for 4 weeks after subculture.

Medium	MT	MS	WPM	GD	B5
Cell Weight(g)*	5.95	4.81	2.99	3.68	3.02

* Mean of triplicates

Table 2. Effect of sucrose concentration in the culture medium on the growth of callus cells. The cells were cultured on each medium for 4 weeks after subculture.

Sucrose Concentration(g/l)	10	20	30	40	50
Cell Weight(g)*	2.80	3.26	4.33	4.00	3.35

* Mean of triplicates

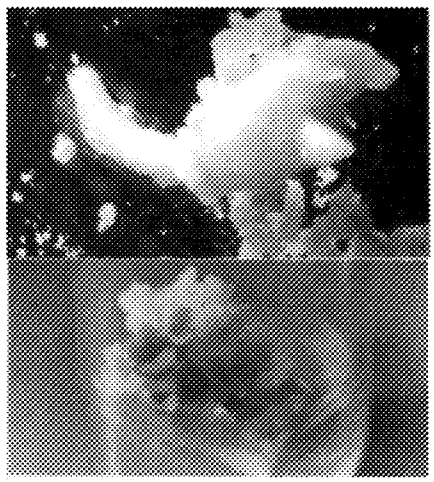

Fig. 1. Somatic embryo (upper) and shoots (lower) formed from callus cells cultured on MT medium containing 10 mg/l of NAA and 0.1 mg of thidiazuron.

Shoot induction was mainly affected by the concentration of NAA in the medium. Shoot formation was increased with the concentration of NAA up to 10 mg/l. The effect of thidiazuron on the shoot formation was relatively low. The highest frequency of shoot formation was obtained on the medium supplemented with 10 mg/l of NAA and 0.1 mg/l of thidiazuron (Table 3).

Table 3. Effects of the concentration of NAA and thidiazuron on shoot formation.

Thidiazuron(mg/l)	NAA(mg/l)				
	0	0.1	1	5	10
0	S	--	SS	S	--
0.001	--	--	--	--	SS
0.01	--	--	--	S	--
0.05	--	--	--	--	--
0.1	--	--	--	S	SSS
0.5	--	--	SS	--	S

-- no shoot formed, S shoot formed

The susceptibility of the cells to kanamycin was tested to find the optimum concentration of the antibiotic in the medium for selecting transformants with the kanamycin resistant gene, nptII. At the level of 50 mg/l of kanamycin the growth of cells was inhibited, but several clones showed continuous growth (Fig. 2, middle).

The growth of the callus cells was stopped at the level of 100 mg/l of kanamycin in the medium, which might be suitable for screening purpose (Fig. 2, upper).

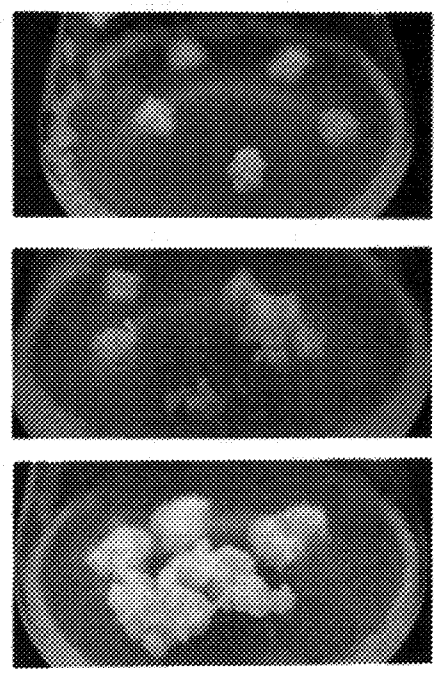

Fig. 2. Growth of callus on the medium with different level of kanamycin. Upper : 100 mg/l, Middle : 50 mg/l, Lower : 0 mg/l.

The efficiency of DNA introduction using the microprojectile bombardment DNA delivery system is affected by many parameters. Among these the pressure of helium gas, the target distance and the gap distance were known to be most important factors.

Although several reasonable GUS scores were obtained from the cells bombarded at higher helium pressures of 1100 and 1300 psi, it was difficult to obtain consistent results. At the low helium pressure condition of 900 psi, consistently high GUS scores were obtained. With short gap distance of 1/8" and short target distance of 3.8 cm, the cells were blown out from the petridish during bombardment.

The highest GUS score was obtained when the cells were bombarded with 900 psi of helium gas pressure, 1/4" of gap distance and 7.0 cm of target distance (Table 4). Therefore this bombardment condition was considered to be optimum for introducing DNA into callus cells of navel orange.

Table 4. Effects of helium pressure, gap distance and target distance on the efficiency of DNA introduction into cells.

Helium Pressure (psi)	Gap Distance (inch)	Target Distance (cm)	GUS Score
900	1/8	3.8	+ + +
	1/8	3.8	+ + +
	1/8	3.8	+ + + +
	1/4	7.0	+ + + +
	1/4	7.0	+ + + +
	1/4	7.0	+ +
	3/8	10.2	+ + + +
	3/8	10.2	+ +
	3/8	10.2	+ + + +
1100	1/8	3.8	+
	1/8	3.8	+ +
	1/8	3.8	+ + +
	1/4	7.0	+ + +
	1/4	7.0	+ +
	1/4	7.0	+
	3/8	10.2	+ + +
	3/8	10.2	+
	3/8	10.2	+
1300	1/8	3.8	+ +
	1/8	3.8	+ +
	1/8	3.8	+ + +
	1/4	7.0	
	1/4	7.0	+ + +
	1/4	7.0	+ +
	3/8	10.2	+
	3/8	10.2	+
	3/8	10.2	+

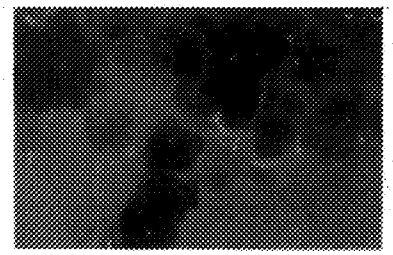

Fig. 3. Expression of the introduced gene in the callus cells bombarded with pBI121 carrying GUS gene.

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