Transformation of Callus Cells of *Citrus sinensis* Osbeck 'Yoshida' with Fatty Acid Desaturase Genes by Microprojectile Bombardment

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ABSTRACT

We optimised the conditions for DNA introduction into callus cells of *Citrus sinensis* Osbeck 'Yoshida' using microprojectile bombardment, and transformed the cells with two kinds of genes, fad3 and fad7, which are related to fatty acid desaturation. The highest transformation efficiency was obtained when the callus cells were bombarded with the pBI121 coated-M17 tungsten particles at 1100 psi of helium pressure, 1/4 inch of gap distance, 6.0 ㎝ of target distance and 27 inch Hg of chamber vacuum. The cells bombarded with the DNA of each gene were cultured on a selective medium containing 100 ㎎/ℓ of kanamycin. We could obtain citrus callus clones which contain DNA's of the above two target genes.

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INTRODUCTION

Fatty acid desaturases introduce double bonds into fatty acids and the genes of desaturases are known to be involved in tolerance of plant against temperature stress. Many reports have revealed that modifications of the fatty acid desaturase genes alter compositions of unsaturated fatty acid in cell membranes and change low-temperature resistance of plants. Now low-temperature resistance could be introduced to plants by transformation with desaturase genes(Ishizaki-Nishizawa et al., 1996).

Recently polyunsaturated fatty acids in membrane lipids were proved to be also important in growth, respiration and photosynthesis as well as low-temperature resistance of plants(Tasaka et al., 1996). Therefore introduction of desaturase genes might improve fruit qualities via enhancing these physiological activities of plants especially at low temperature.

In this study we tried to transform citrus with fatty acid desaturase genes for improving qualities of citrus fruits.

MATERIALS AND METHODS

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

Two kinds of fatty acid desaturase genes, fad3 and fad7, were used. Each gene was constructed in a plant expression vector, pB I 121, harboring npt II.

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.

Transformants were screened on a selective MT medium containing 100㎎/ℓ of kanamycin. The introduced DNA of each gene was identified by PCR.

RESULTS AND DISCUSSION

The efficiency of DNA introduction using the microprojectile bombardment DNA delivery system is known to be affected by many parameters including cell types, carrier particle types, helium gas pressure, target distance and gap distance etc. Here we tested the effects of carrier particle types and helium gas pressure on the efficiency of DNA introduction into citrus callus cells. Table 1 is a summary of the results.

The cells bombarded with gold particles showed relatively high GUS scores. The highest GUS score was obtained when the cells were bombarded with tungsten M17 particles at 1100 psi of helium gas pressure, 1/4" of gap distance and 6.0 ㎝ of target distance. Gold particles are more expensive than tungsten and tungsten particles did not showed obvious toxic effects on the growth of citrus cells. Therefore we used tungsten M17 particles for further experiments. The GUS gene introduced in citrus callus cells as a reporter was succesfully expressed(Fig. 1).

Table 1. Effects of microprojectile particle type and helium pressure on efficiency of DNA introduction into citrus callus cells.

Material of particle	Type of particle	Helium Pressure(psi)	GUS score
Tungsten	M5	900	+
		1100	+
		1300	+ + +
	M10	900	+ + +
		1100	+ +
		1300	+
	M17	900	+ + +
		1100	+ + + +
		1300	+ +
	M20	900	+
		1100	+
		1300	+
	M25	900	−
		1100	−
		1300	+ + +
Gold	1.1㎛	900	+ + +
		1100	+ +
		1300	+ +

Note : The gap distance, target distance and chamber vacuum were 1/4 inch, 6.0 ㎝ and 27 inch Hg, respectively. The plasmid pBI121 harboring GUS gene was used for evaluating efficiency of DNA introduction into cells.

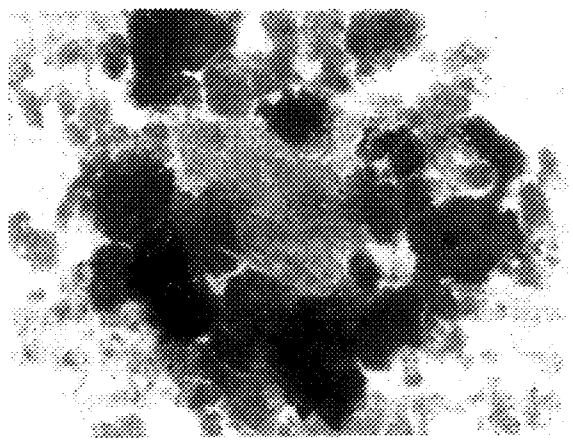

Fig. 1. Transient expressions of GUS gene in the citrus callus cells after introduction of pBI121.

The citrus callus cells bombarded with pBI121 harboring both of nptII and target gene, fad3 or fad7, were screened on a MT medium containing 100 ㎎/ℓ of kanamycin. Some cells grew on the selective medium to form callus clusters(Fig. 2). The DNA's extracted from the selected citrus clones were amplified by PCR with the specific primers for nptII or target desaturase genes to confirm that the clones contain the introduced DNA's.

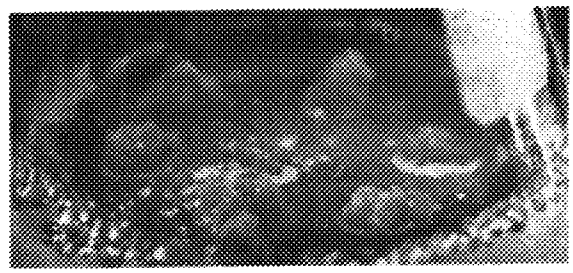

Fig. 2. Selective growths of citrus callus cells clones on MT medium containing kanamycin after bombardment with pBI121 containing both of nptII and desaturase gene.

The expected DNA bands of 795 bp for nptII were observed in two (lane 5 and 6) among four citrus clones from the cells bombarded with pBI121harboring nptII and fad3, while the other two(lane 7 and 8) did not showed the nptII DNA band (Fig. 2. Upper). The 700 bp DNA bands for fad3 were also observed in the two clones which had the 795 bp DNA band. Therefore these clones were proven to contain both genes of nptII and fad3. The clones without nptII gene did not have fad3 gene either.

Among ten clones from the cells bombarded with pBI121 containing both of nptII and fad7 genes, five clones(lane 5, 6, 9, 13 and 14) showed positive DNA bands of 795 bp for nptII gene. Although the DNA for fad7 gene was not analized here, these clones with nptII genes supposed to contain fad7 genes. Because the fad7 gene was constructed simultaneously in the same plasmid, pBI121, harboring the nptII gene.

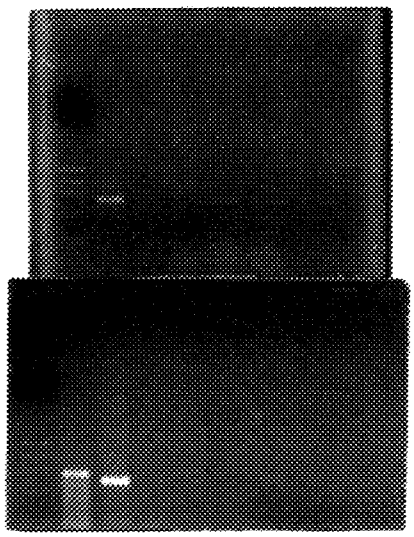

Fig. 3. PCR-amplified DNA's of nptII and fad3 genes from the clones bombarded with pBI121 containing both of nptII and fad3.

Upper(nptII) : lane 1 : size marker, 2 : positive control(+nptII DNA), 3 : negative control(-nptII DNA), 4 : non-bombarded cells, 5-8 : clones bombarded with nptII-fad3.

Lower(fad3) : lane 1 : marker, 2 : positive control(+nptII DNA), 3 : positive control(+fad3 DNA), 4 : negative control(-nptII DNA), 5 : negative control(-fad3 DNA), 6 : non-bombarded cells, 7-10 : clones bombarded with nptII-fad3.

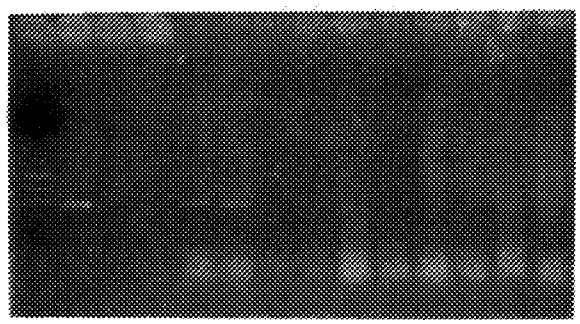

Fig. 4. PCR-amplified DNA's of nptII gene from the clones bombarded with pBI121 containing both of nptII and fad7.

lane 1 : size marker, 2 : positive control(+nptII DNA), 3 : negative control(-nptII DNA), 4 : non-bombarded cells, 5-15 : clones bombarded with nptII-fad7.

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