TRANSFORMATION OF *CITRUS* BY MICROPROJECTILE BOMBARDMENT

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ABSTRACT

The conditions for plant regeneration from tissue sections or callus cells of *Citrus* species and introduction of DNA by microprojectile bombardment were studied. The plantlets were sucessfully regenerated from both tissue segments and callus cells. The direct shootings from sections of stems and cotyledons occured in two species of Citrus, especially multi-shoots were formed from stem sections of 'sambokam' on Gresshoff and Doy (GD) medium containing 1mg/l of BA. The plantlets could be also obtained from the embryogenic callus cells derived from unfertilized and undeveloped seeds of *Citrus unshiu* on Murashige and Thucker (MT) medium containing auxin. The transient expression study by using GUS gene showed that the type of vector plasmid and target distance affected the transformation efficiency. Among the tested plasmids, pBI221 gave the highest GUS score. The optimum target distance was 7 cm.

INTRODUCTION

Citrus species are very important in agriculture of Cheju. Their improvement has been mainly based on mutations and clonal selections. Breeding of satsumas (*Citrus unshiu*), the most important species in Cheju, has been difficult to achieve due to polyembryony and male sterility. The difficulty in breeding of this citrus species can be overcome by using transgenic plant technique. In this study microprojectile bombardment system was used as a method for transformation of citrus species.

For the successful construction of transgenic plant, establishment of condition for plant regeneration is a prerequisite. We studied first two systems of plant regeneration via organogenesis and embryogenesis.

A foregin DNA can be introduced into plant cells by microprojectile bombardment. The efficiency of transformation is affected by several factors including type of cells, type of vector plasmid carryng target gene and various conditions of bombardment. In this paper the conditions for introduction of a gene into citrus cells by helium pressure derived microprojectile bombardment system was discussed.

MATERIALS AND METHODS

Citrus unshiu, *C. junos* and *C. sambokam* were used as plant materials. Cotyledon, leaf and stem sections of seedlings from seeds germinated on MS basal medium were used for organogenesis. Embryogenic callus cells were prepared according to the method established by Dr. N. Nito (Saga University, Japan). PDS-1000/He Biolistic Particle Delivery System (Bio-Rad) was used for bombardment. GUS gene was used for transient expression study to assess the transformation efficiency.

RESULTS AND DISCUSSION

In general, source of explant is very important for the sucessful organogenesis from cultured plant tissue. Although the best source of explant is plantlets germinated from seeds under sterile condition, in case of *C. unshiu* we used explants excised from plants grown under field condition: there are no seed in the fruit of this species due to polyembryony and male sterility.

Neither shoot nor root was formed from the explants of *C. unshiu* cultured on the tested media. Only callus was induced from leaf and stem sections cultured on MS and WPM medium (Table 1).

Table 1. Effects of culture media on organogenesis from cotyledon, leaf and stem sections of *Citrus* species.

	MS	WPM	NN	GD
C. unshiu				
cotyledon	-	-	-	-
leaf	C	C		
stem	C	C	-	-
C. junos				
cotyledon	-	CRS	-	-
leaf	C	CRS	-	-
stem	C	CRS	-	-
C. sambokam				
cotyledon	-	-	-	-
leaf	-	C	-	C
stem	-	C	-	CS

Each medium was supplemented with 0.5mg/l of NAA and 0.5mg/l of BA. C, R and S designated for formation of callus, root and shoot, respectively.

Explants of *C. junos* formed both of shoot and root when cultured on WPM medium. On MS medium, only callus was induced. In case of *C. sambokam* shoots were formed from stem sections cultured on GD medium, and callus was formed from leaf and stem sections cultured on WPM and GD medium (Fig.1).

Growth regulators in the culture medium affected organogenesis, low level of NAA induced mainly shoots while the high level of this regulator induced roots and calli (Table. 2).

Plant regeneration can be also achieved via embryogenesis. As mentioned above *C. unshiu* is polyembryonic and male sterile, but there are unfertilized and undeveloped seeds in the fruit. N. Nito reported that embryogenic callus cells could be induced from immature seeds under specific tissue culture condition. We obtained shoots from the embryogenic callus cells on

Fig. 1. Shoots regenerated by organogenesis from the stem section of *C. sambogam* cultured on MS medium containing 1mg/l of BA.

Table 2. Effects of NAA and BA in culture medium on organogenesis from cotyledon, leaf and stem sections of *C. junos*.

			NAA(mg/l)				
			0	0.1	1	5	10
BA (mg/ml)	0	cotyledon	-	-	-	-	C
		leaf	-	-	-	R	C
		stem	S	S	R	C	C
	0.1	cotyledon	C	-	-	CR	CR
		leaf	-	-	C	CR	CR
		stem	C	C	C	CR	C
	1	cotyledon	-	SR	-	C	-
		leaf	-	R	-	-	-
		stem	S	SR	C	-	-
	5	cotyledon	-	C	C	C	-
		leaf	-	-	-	-	-
		stem	S	S	S	-	C
	10	cotyledon	-	-	-	-	C
		leaf	-	-	-	-	-
		stem	S	-	C	C	C

MS was used as a culture medium. C, R and S designated for formation of callus, root and shoot, respectively.

MT medium containing 1mg/l of NAA (Fig. 2).

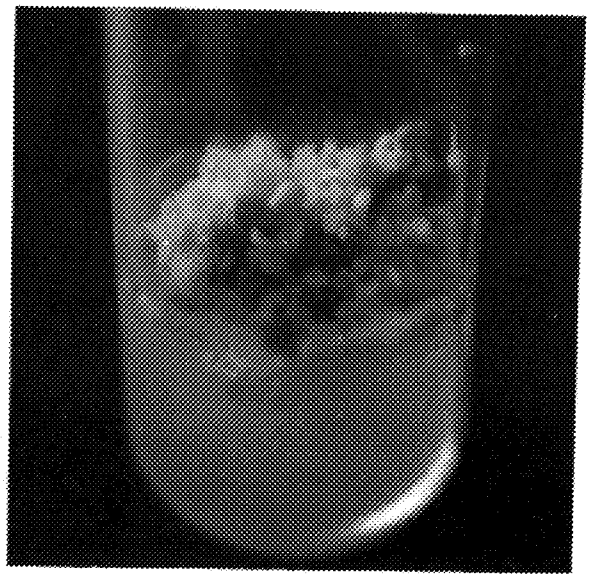

Fig. 2. Shoots regenerated by embryogenesis from the calus cells derived from the unfertilized and undeveloped seeds of *C. sambogam*

The embryogenic cells were subcultured every 10 days under light condition on MT medium containing 10mg/l of BA.

Table 3 shows that the type of plasmid affected efficiency. Among the three tested plamids, pBI121, pBI221 and pBarGUS, pBI221 gaved the highest GUS score.

Target distance is one of the most important parameters in introduction of DNA into cells by microprojectile bombardment. In case of stem section of citrus, the

Table 3. Effect of type of plasmid carrying target gene on transformation efficiency.

Micro-particle (tungsten)	Helium pressure (psi)	Target distance (cm)	Plasmid	GUS score
M17	1300	3.8	pBI121	++
M17	1300	3.8	pBI221	+++
M17	1300	3.8	pBarGUS	-

GUS score was used as a transformation efficiency.

Table 4. Effect of target distance on transformation efficiency.

Micro-particle (tungsten)	Helium pressure (psi)	Target distance (cm)	Plasmid	GUS score
M17	1300	3.8	pBI121	++
M17	1300	7.0	pBI121	+++
M17	1300	10.2	pBI121	-

GUS score was used as a transformation efficiency.

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