Tissue Culture and Transformation of Rice (*Oryza sativa* L.) using Tobacco Nurse Cells.

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Abstract

Plant genetic engineering involves delivery and integration of foreign genes into individual plant cells and subsequent regeneration of transformed cells into whole plants so that the newly acquired genes can be inherited in the progeny. Gene delivery systems such as PEG-mediated uptake of DNA by protoplasts, biolistics and quite recently the *Agrobacterium*-mediated gene transfer technique have been used in genetic engineering of cereals. However, regeneration of plants and low transformation frequency are still limiting factors in successful cereal transformation. In this project, using rice as a model plant, methods have been developed to improve regeneration frequency and to enhance the efficiency of *Agrobacterium* mediated transformation technique.

The use of fast growing cell lines as nurse culture is a common practice in regeneration of plants from protoplasts. In this project the heterologous system of tobacco nurse cells was utilised to improve the regeneration of suspension cells of two rice cultivars. In the nurse culture treatment, more than 50% of green calli gave rise to plantlets, against 13-20% in the control. The overall plant regeneration frequencies in cv. 77-170 and cv. Nipponbare were 41% and 38% in the nurse culture treated calli and 5% and 4% in the control calli, respectively. The plant regeneration frequency of both rice cultivars was increased by 8-9 times using tobacco nurse cells. Multiple shoot formation was commonly seen in the tobacco nurse cell treated calli, while control calli mainly formed single shoots.

Subsequently, the effects of tobacco nurse cells on *Agrobacterium* mediated transformation of rice callus were evaluated. Two strains of *Agrobacterium* namely AGL1 and LBA4404 containing the plasmid pIG121Hm or pPCV707+Gus were used. The calli used were derived from scutellum of immature embryos of three rice cultivars: Nipponbare, 77-170

and T-309. The co-cultivation of rice calli with *Agrobacterium* was carried out on NB medium, NB medium with *acetosyringone* or NB medium with tobacco nurse cells. The tobacco nurse cells enhanced the recovery of embryogenic properties of rice calli after co-cultivation with *Agrobacterium*. The recovery of embryogenic calli was as high as 100% after co-cultivation on tobacco nurse cells, while it was reduced significantly (down to 27% in cv. T-309) when nurse cells were excluded from the co-cultivation medium. Co-cultivation on tobacco nurse cells increased the transformation frequency in all three rice cultivars. The number of hygromycin resistant rice calli and GUS expressing calli indicated an interaction between the rice cultivars and the *Agrobacterium* strain-plasmid combinations used. The *Agrobacterium* strain LBA4404 (pIG121Hm) was more effective with cv. 77-170, while AGL1 (pIG121Hm) produced high transformation frequencies in cultivars Nipponbare and T-309. Transformed rice calli were obtained even when *acetosyringone* and tobacco nurse cells were excluded from the co-cultivation medium, albeit at a much lower frequency. The presence of *uidA* gene in potentially transformed rice calli was confirmed by PCR analysis.

In this study tobacco nurse cells enhanced the regeneration frequency of long term suspension cells and *Agrobacterium* mediated transformation of rice. These findings suggest scope for utilising the tobacco nurse culture technique with other economically important cereal crops to improve regeneration frequency and *Agrobacterium* mediated transformation.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed: _ **Date:** 30.9.97

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Abbreviations

ABA	abscisic acid
BAP	benzylaminopurine
bp	base pair
C	centigrade
$CaCl_2$	calcium chloride
cm	centimetre
CsCl	cesium chloride
cv.	cultivar
dNTP's	deoxyribonucleoside triphosphates
DNA	deoxyribonucleic acid
DNAase	deoxyribonuclease
ds	double stranded
E. coli	*Escherichia coli*
Na_2-EDTA	disodium ethylenediaminetetraacetate
g	grams
GUS	β-glucuronidase
IAA	indole-3-acetic acid
kbp	kilo base pair
L	liter
M	molar
mg	milligrams
$MgCl_2$	magnesium chloride
ml	milliliter
µg	microgram

µl	microliter
µM	micromolar
NAA	napthalene acetic acid
NaCl	sodium chloride
NaOAc	sodium acetate
OD	optical density
PCR	polymerase chain reaction
psi	pounds per square inch
RNA	ribonucleic acid
RNAase	ribonuclease
rpm	revolutions per minute
ss	single stranded
Tris	Tris (hydroxymethyl) aminomethane
UV	ultra violet
X-Gluc	5-bromo-4-chloro-3-indolyl-β-D-glucuronide
2,4-D	2,4-dichlorophenoxyacetic acid