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Agrobacterium-mediated gene transfer into kiwifruit

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Abstract

A system has been developed to aid in the establishment of *Agrobacterium*-mediated transformation for new plant species. A series of binary vectors have been constructed that express a chimaeric β-D-glucuronidase (GUS) gene in plants cells but not in bacterial cells. This feature allows GUS activity from transformed plant cells to be assayed in the presence of *Agrobacterium*.

Preliminary experiments examined the expression of these chimaeric GUS genes in transformed petunia leaf discs. GUS expression was detectable 2 days after inoculation, peaked at 3 - 4 days and then declined; if selection was imposed expression increased again after 10 - 14 days. The amount of expression observed 4 days after inoculation correlated well with stable integration as measured by kanamycin resistance, hormone independence, and gall formation. Histochemical staining of inoculated leaf discs confirmed the transient peak of GUS expression 3 - 4 days after inoculation. Surprisingly, GUS expression was concentrated in localized zones on the circumference of the disc; within these zones essentially all the cells appeared to be expressing GUS. These results suggest that the frequency of gene transfer from *Agrobacterium* is extremely high within localized regions of the petunia leaf explants, but that the frequency of stable integration is several orders of magnitude lower.

A reliable *Agrobacterium*-mediated transformation system has been established for kiwifruit (*Actinidia deliciosa* var. *deliciosa* cv. Hayward) by using transient expression of GUS to monitor gene transfer frequencies. *In vitro* culture of kiwifruit plants and conditions for regeneration of plants from leaf discs have been established. Several factors were found to improve gene transfer frequencies in kiwifruit: (i) healthy actively growing source tissue; (ii) the use of *Agrobacterium* strain A281; (iii) the presence of a layer of moistened filter paper between the leaf explants and the cocultivation media; and (iv) the presence of 20 μM acetosyringone in both the bacterial culture media and in the cocultivation media. Pre-culture of leaf explants significantly inhibited gene transfer, particularly at the cut edge of the explants.

Using the optimized transformation system, at least one transgenic plant can be regenerated from each leaf inoculated. Stable transformation frequencies have been shown to vary significantly between different binary vectors. Phenotypic, PCR, and Southern analysis has confirmed the presence of stably integrated T-DNA in several transgenic kiwifruit plants.

Table of Contents

Contents i
Acknowledgements vii
Abbreviations ix

Chapter 1 Introduction 1

1.1 The introduction of foreign DNA into plants 1
1.2 Kiwifruit 3
1.3 Plant gene transfer systems 5
1.3.1 *Agrobacterium*-mediated gene transfer 5
1.3.2 Free-DNA delivery 7
1.3.3 Microprojectile-mediated gene transfer 7
1.3.4 Viral vectors 9
1.3.5 Summary 9
1.4 The molecular biology of *Agrobacterium*-mediated gene transfer 10
1.4.1 Signal perception 11
1.4.2 Binding of *Agrobacterium* to plant cells 13
1.4.3 Generation of the transferred DNA 14
1.4.4 Transfer of the T-DNA complex 16
1.4.5 Other virulence related loci 17
1.4.6 Integration of the T-DNA 17
1.4.7 The development of *Agrobacterium*-mediated gene transfer vectors 18
Cointegrate vectors 18
Binary vectors 19
Plant expressible marker genes 20
Expression of the marker gene 20
1.5 The aim of this research 21

Chapter 2 Materials and methods 23
2.1 Bacterial strains 23
2.2 Plant species 24
2.3 Antibiotics 24
2.4 Buffers and solutions 24
2.5 Oligonucleotides 25
2.6 Enzymes and radiochemicals 26
2.7 Bacterial growth media 26
2.7.1 *E. coli* growth media 26
2.7.2 *Agrobacterium* growth media 26
2.8 Plant growth media 26
2.8.1 Kiwifruit growth media 26
2.8.2 Petunia growth media 27
2.8.3 Tomato suspension media 27
2.9 Manipulation of DNA 27
2.9.1 Plasmid DNA extraction 27
2.9.1.1 Alkaline lysis extraction 27
2.9.1.2 CTAB extraction 28
2.9.1.3 Small scale plasmid preparation 28
2.9.1.4 Preparation of plasmid DNA from *Agrobacterium* 28
2.9.1.5 Purification of plasmid DNA on CsCl-Ethidium bromide gradients 28
2.9.2 Preparation of kiwifruit genomic DNA 29
2.9.2.1 Purification of genomic DNA by sucrose gradient centrifugation 29
2.9.3 Agarose gel electrophoresis 30
2.9.3.1 Isolation of DNA fragments by electroelution 30
2.9.4 Restriction endonuclease digestion of DNA 30
2.9.5 Filling uneven restriction ends with T4 DNA polymerase 31
2.9.6 Dephosphorylation of DNA 31
2.9.7 Ligation of DNA fragments 31
2.9.8 DNA Sequencing 32
2.9.9 Polymerase chain reaction (PCR) 32
2.9.10 Southern analysis 32
2.9.10.1 Transfer 32
2.9.10.2 Digoxygenin labelling of probe DNA 33
2.9.10.3 Hybridization and washing procedures 33
2.9.10.4 Detection of hybridized probe 33
2.9.10.5 Removal of probe and reuse of membranes 34
2.10 Manipulation of bacteria 34

2.10.1 Bacterial transformation 34
2.10.1.1 Preparation of competent cells 34
2.10.1.2 Transformation 35
2.10.2 Screening of bacteria for expression of lacα 35
2.10.3 Tri-parental mating 35
2.11 Plant Growth conditions 35
2.11.1 Surface sterilization of plant material 36
2.11.2 Establishment of *in vitro* stocks of kiwifruit 36
2.11.3 Maintenance of *in vitro* stocks of kiwifruit 36
2.11.4 Petunia growth conditions 36
2.11.5 Tomato suspension cultures 37
2.12 Transformation of petunia 37
2.13 *Agrobacterium*-mediated transformation of kiwifruit 38
2.14 Microprojectile-mediated gene transfer into kiwifruit 39
2.15 GUS assays 40
2.15.1 Fluorescent GUS assay 40
2.15.2 Histochemical GUS assay 40
2.16 Software 41
Chapter 3 Construction and preliminary characterization of GUS expression vectors 43
3.1 Introduction 43
3.2 Construction of GUS expression vectors 43
3.2.1 pKIWI100 and pKIWI101 44
3.2.2 pKIWI113 and pKIWI114 47
3.3 Binary vector construction 47
3.3.1 pKIWI103 and pKIWI104 47
3.3.2 pKIWI105 and pKIWI106 47
3.3.3 pKIWI109 48
3.3.4 pKIWI110 and pKIWI111 49
3.4 Transfer of binary vectors to *Agrobacterium* strains 50
3.5 Expression of the reporter gene construct in bacteria 52
3.6 Expression of the reporter gene construct in petunia 53
3.7 Correlation of histochemical and fluorescent GUS assays 55
3.8 Inhibition of GUS activity by saccharic acid 1-4 lactone 55
3.9 Transfer of the GUS gene is required for expression of GUS activity 56
3.10 Comparison of binary GUS expression vectors 57
3.11 Discussion 58

Chapter 4 Gene transfer in petunia 61
4.1 Introduction 61
4.2 Early detection of GUS expression 61
4.3 Time course of GUS expression after gene transfer 62
4.4 Localization of GUS expression 64
4.5 Relationship of early GUS expression to stable integration 68
4.6 Optimization of gene transfer by monitoring early GUS expression 70
4.6.1 Concentration of *Agrobacterium* 70
4.6.2 Growth state of *Agrobacterium* 71
4.6.3 Duration of the co-cultivation period 72
4.6.4 Pre-culture of explants 73
4.7 Discussion 74
4.7.1 Transient expression of GUS after *Agrobacterium*-mediated gene transfer 75
4.7.2 Local regions of "enhanced gene transfer" 75
4.7.3 Correlation of transformation assays 76
4.7.4 Optimization of gene transfer in petunia 77
4.7.5 Summary 78
Chapter 5 Transformation of kiwifruit 81
5.1 Introduction 81
5.2 *In vitro* culture of kiwifruit 82
5.2.1 Establishment and maintenance of *in vitro* stocks of kiwifruit 82
Supplemental media 83
Media support 84
Contamination during *in vitro* culture 86
5.3 Regeneration of plants from tissue explants 87
Hormones 87
Leaf colour, size, and positional origin of explant 90
Agar vs Phytagel™ vs Gelrite™ 91
5.4 Microprojectile-mediated gene transfer into kiwifruit 91
5.5 Production of galls by *Agrobacterium* 93
5.6 *Agrobacterium*-mediated gene transfer into kiwifruit 95
5.6.1 Tissue type 95
Roots, stems, and leaves 96
Seedlings, cotyledons, and hypocotyls 96
5.6.2 Comparison of *Agrobacterium* strains 97
5.6.3 Comparison of *Actinidia* species 99
5.6.4 Optimization of gene transfer 100
5.6.4.1 Plant factors 100

5.6.4.2 Bacterial factors 101
Acetosyringone 102
Opines 104
5.6.4.3 Inoculation and co-cultivation conditions 105
Filter paper 106
Exogenously applied hormones 107
Gelling agent 108
Moisture content 109
Blotting and rinsing 110
Pre-culture of the kiwifruit leaf explants 111
5.6.5 Localization of gene transfer in kiwifruit 112
5.6.6 Size of T-DNA: pKIWI110 vs pKIWI109 vs pKIWI105 113
5.7 Stable transformation of kiwifruit 115
5.7.1 Stable integration and selection of transformants 115
Yield 116
Timing of kanamycin selection 118
5.7.2 Comparison of binary vectors pGA643, pLAN421, pBI101.2, and pKIWI110 118
5.8 Analysis of putative transgenic plants 122
5.8.1 Phenotypic analysis 122
5.8.2 PCR analysis 123
5.8.3 Southern analysis 125
5.8.4 Structure of the integrated T-DNA for twelve transgenic kiwifruit 129
5.9 Conclusions and discussion 132
5.9.1 *In vitro* culture of kiwifruit 132
5.9.2 Regeneration of kiwifruit leaf explants 133
5.9.3 The use of transient expression to develop kiwifruit transformation 134
5.9.4 The use of virulent *Agrobacterium* strains 135
5.9.5 Production of transgenic plants 136
5.9.6 Analysis of transgenic plants 136
Chapter 6 Concluding discussion 139
6.1 The Vectors 139
6.2 *Agrobacterium*-mediated gene transfer in petunia 140
6.2.1 Localization of gene transfer 140
6.2.2 Transient expression from the T-DNA 142
6.2.3 Further experiments 144
6.3 *Agrobacterium*-mediated transformation of kiwifruit 145
6.4 *Agrobacterium*-mediated transformation in general 147

6.5 Future directions for gene transfer in kiwifruit 149

References 151

Appendix 1 Kiwifruit transformation experiments: conditions and results I

Appendix 2 The complete nucleotide sequence of pKIWI101 XVII

Appendix 3 Publication XXI

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Abbreviations

2,4-D	2,4-Dichlorophyenoxyacetic acid
35S	35S promoter region of Cauliflower mosaic virus
2ip	6-(γ,γ-Dimethylallylamino)-purine
6BA	6-Benzylaminopurine
$A_{x\ nm}$	Absorbance at x nanometres
AS	acetosyringone
bp	base pairs
CaMV	Cauliflower mosaic virus
CTAB	cetyl-trimethylammonium bromide
°C	degrees celcius
cv.	cultivar
dATP	2'-deoxyadenosine triphosphate
dCTP	2'-deoxycytadine triphosphate
dGTP	2'-deoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
dNTP	deoxynucleotides
ds	double-stranded
DSIR	department of Scientific and Industrial Research
DTT	Dithiothreitol
EDTA	ethylenediamine tetraacetic acid
EtBr	Ethidium bromide
GUS	β-D-glucuronidase
h	hours
IAA	Indole-3-acteic acid
kbp	kilobase pairs
kin	Kinetin
min	minutes

MU	4-methylumbelliferone
MUG	4-methylumbelliferyl-β-D-glucuronide
NAA	α-naphthalene acetic acid
ocs	octopine synthase polyadenylation region
PEG	polyethylene glycol
rATP	adenosine triphosphate
Ri	Root inducing
RNA'se	Ribonuclease
rpm	revolutions per minute
r.t.	room temperature
s	seconds
SDS	Sodium dodecylsulfate
ss	single-stranded
T-DNA	transferred DNA
Ti	Tumour-inducing
Tris	Tris(hydroxymethyl)-aminomethane
TTP	thymadine 5'-triphosphate
UTP	uracil triphosphate
UV	ultraviolet
var.	variety
x g	times the force of gravity (with respect to centrifugation)
X-gluc	5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid
YN	Yeast nutrient