Molecular Approaches to Horticultural Crop Improvement

Ross G. Atkinson

Plant Science

School of Biological Sciences

University of Auckland

Thesis submitted in fulfilment

of the requirements for

the degree of

Doctor of Philosophy

May, 1993

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Acknowledgements

I would like to thank the following people and organisations:

My supervisor, Associate Professor Richard Gardner for his guidance and help during the research undertaken in this thesis.

The Horticultural and Food Research Institute of New Zealand Ltd (previously DSIR Fruit and Trees, previously DSIR Division of Horticulture and Processing) for allowing me study leave to undertake this research and for the financial support to do so.

My Hort Research PhD advisers Alan Seal, Don Burns and Paul Glucina for their interest and advice.

Keith Hammett, Greg Pringle, Mark McNeilage and Allan White for providing plant material and Nga Tama and Sigrun Steinhagen for looking after my plants in the glasshouse.

Elspeth MacRae for the use of her kiwifruit cDNA library and mRNA samples.

Debbie Hutching for all the help with the transformation experiments.

Amanda Chapman for doing all those sequencing runs.

Dave Whittaker for collaboration with cloning and sequencing of PG clones.

Bart Janssen for letting me use the pKIWI110 vector and for all the advice and ideas.

Jeannette Keeling for help with the RNA preps, the λ preps and everything else I've borrowed or used over the years.

My Hort Research colleagues based at University - Lena, Trixie and Jacqui; and the PMBies - Susan, Kim, Jeannette, Keith, Colin, Robin, Tom, Andreas, Lynn and Gwen for their advice, friendship and support and for making the PMB lab such an enjoyable place to work.

Susan, Kim and Jeannette for their diligence in proof-reading this manuscript.

Astrid for her constant supply of enthusiasm (and calories).

Finally, I would especially like to thank my family for their tolerance, support and understanding throughout the course of this work.

Abstract

Plant biotechnology and molecular biology are now being used to complement conventional breeding programmes in most of the world's important crop species. The aim of the research described in this thesis was to improve New Zealand's horticultural crop plants through application of similar molecular techniques.

A) Gene transfer systems for apple, pepino and tamarillo

Tissue culture systems were developed for micropropagation and regeneration of apple (cv Royal Gala), pepino (cv El Camino) and tamarillo (selection Oratia Red). In all three species, transient expression of the *gus*A reporter gene was observed and kanamycin resistant callus was produced, following inoculation with the pKIWI110 binary vector in the avirulent *Agrobacterium* strain LBA4404. No transgenic apple shoots were obtained. However, transgenic pepino and tamarillo plants expressing the *gus*A reporter gene, kanamycin resistance and herbicide tolerance were successfully regenerated. PCR and Southern analysis provided molecular evidence for integration of foreign DNA into the genomes of transgenic pepino and tamarillo plants, but indicated deletions of the integrated T-DNAs had occurred with high frequency. Inheritance of the transgenic phenotypes was demonstrated in the progeny of transgenic tamarillo plants.

B) Characterisation of polygalacturonase genes

A kiwifruit genomic clone with homology to a tomato cDNA clone for polygalacturonase (PG) was sequenced over an 8.1 kb region. The sequence revealed a gene divided into nine exons, with 58% overall identity to the tomato PG gene at the amino acid level. Significant homology was also noted to PG genes isolated from peach, *Oenothera organensis* and maize, particularly in several blocks of conserved amino acids believed to encode the active site of the enzyme. Analysis of the kiwifruit PG promoter revealed three 81 bp direct repeat sequences just upstream of the kiwifruit peptide start codon. These repeats were also conserved in a second kiwifruit PG genomic clone. Characterisation of partial cDNA clones indicated that at least two mRNAs for PG were expressed in ripe kiwifruit. Southern hybridisation detected the PG gene at low copy number in the genomes of kiwifruit, two other *Actinidia* species, apple and pepino. PCR was used to amplify a fragment of the apple PG gene for sequence analysis.

PG sequences were also used to help define the genetic origin of kiwifruit. A region of the PG gene was amplified and sequenced from four *Actinidia* species: kiwifruit (*A. deliciosa*), *A. chinensis*, *A. eriantha* and *A. chrysantha*. These sequences were used to produce a phylogeny using PAUP

(phylogenetic analysis using parsimony). Two distinct lineages of PG genes were observed in the genomes of *A. deliciosa, A. chinensis* and *A. eriantha.* Within both these lineages, *A. deliciosa* sequences were quite distinct to those found in the other three *Actinidia* species, with the exception of a single sequence that was identical to *A. chinensis.* These results suggest that hexaploid kiwifruit is an allopolyploid with *A. chinensis* and at least one other *Actinidia* species as likely progenitors.

Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
35S	35S promoter region of cauliflower mosaic virus
2-iP	isopentenyl adenine
ACC	1-aminocyclopropane-1-carboxylic acid
als	acetolactate synthase
BA	6-benzylaminopurine
bma	basal medium solidified with 0.8% Davis agar
bmp	basal medium solidified with 0.2% phytagel
bp	base pair(s)
Bt	*Bacillus thuringiensis*
BRL	Bethesda Research Laboratories
°C	degrees Celsius
cDNA	complementary DNA
CTAB	cetyl-trimethylammonium bromide
cv(s)	cultivar(s)
dCTP	2'-deoxycytidine triphosphate
dNTP(s)	deoxynucleotide triphosphate(s)
DIG	digoxygenin
DMDC	dimethyldicarbonate
DNA	deoxyribonucleic acid
DSIR	Department of Scientific and Industrial Research
DTT	dithiothriotol
EDTA	ethylene diamine tetra-acetic acid
EtBr	ethidium bromide
g	acceleration of gravity
GA_3	gibberellic acid
*gus*A	β-D-glucuronidase A
h	hour(s)
Hort Research	The Horticultural and Food Research Institute of New Zealand Ltd
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
ICMP	International Collection of Microorganisms from Plants
IPTG	isopropyl-β-D-thiogalactoside
kb	kilobase(s)
mRNA	messenger RNA

min	minute(s)
NAA	naphthaleneacetic acid
*npt*II	neomycin phosphotransferase II
NZ	New Zealand
PAUP	phylogenetic analysis using parsimony
PCR	polymerase chain reaction
PEG	polyethylene glycol
PG	polygalacturonase
rATP	adenosine triphosphate
RFLP(s)	restriction fragment length polymorphism(s)
RNA	ribonucleic acid
rpm	revolutions per minute
s	second(s)
SDS	sodium dodecyl sulphate
TaMV	tamarillo mosaic virus
T-DNA	transferred DNA
Ti	tumour-inducing
Tris.Cl	Tris (hydroxymethyl)-aminomethane
tRNA	transfer RNA
UWGCG	University of Wisconsin Genetics Computer Group
var	variety
vir	virulence
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-thiogalactoside
X-Gluc	5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid