Molecular characterisation of *Eucalyptus grandis* PGIP

by

Raksha Bhoora

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Study Leader
Prof. B.D. Wingfield

Co-study Leaders
Prof. D.K. Berger
Dr. P.M. Chimwamurombe
Prof. M.J. Wingfield

Dedicated to my late father

Vasantrai Bhoora

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List Of Abbreviations

AA	amino acids
Amp^R	ampicillin resistance
ADP1	adaptor primer 1
ADP2	adaptor primer 2
ADA	agarose diffusion assay
ARC	Agricultural Research Council
Avr	avirulence
BAP	6-Benzylaminopurine
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
bp	base pair
BSA	bovine serum albumin
CAT	chloroamphenicol acetyltransferase
CAMV	cauliflower mosaic virus
cDNA	complementary DNA
CWDE	cell wall-degrading enzymes
cv	cultivar
dH_2O	distilled water
ddH_2O	double distilled water
DEB	DNA extraction buffer
DIG	digoxygenin
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EDTA	ethylenediamine tetraacetic acid
endo-PGs	endopolygalacturonase
EtOH	ethanol
GUS	β-glucuronidase
hpt	hygromycin phosphotransferase
IAA	indole-3-Acetic Acid
IEF	isoelectric focusing
IPTG	isopropyl-β-D-thiogalactopyranoside
Kan^R	kanamycin resistance

kb	kilobasepair
kDa	kilodalton
Km	kanamycin
LB	Luria Bertani
LRR	leucine-rich repeat
mRNA	messenger ribonucleic acid
MS	Murashige and Skoog
NAA	1-naphthylacetic acid
NBT	nitroblue tetrazolium chloride
nptII	neomycin phosphotransferase II
ng	nanogram
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PAHBAH	ρ-4-amino-2-hydroxybenzoicacid hydrazide
PCR	polymerase chain reaction
PEG	polyethylene glycol
PG	polygalacturonase
PGA	polygalacturonic acid
PGIP	polygalacturonase-inhibiting protein
pI	isoelectric point
PL	pectate lyase
PME	pectin methylesterase
PRI	porcine ribonuclease inhibitor
PS	panhandle structures
PVX	potato virus X
R-genes	resistance genes
RACE	rapid amplification of cDNA ends
Rf	rifampicin
RI	ribonuclease inhibitor
RNA	ribonucleic acid
ss	single stranded
SCN	soybean cyst nematode
SDS	sodium dodecyl sulphate

TAE	Tris-acetate ethylenediamine tetraacetic acid
T-DNA	transferred DNA
TE	Tris ethylenediamine tetraacetic acid
TEV	tobacco etch virus
Ti	tumour inducing
Tm	melting temperature
TNE	Tris-sodium chloride EDTA
UV	ultraviolet
vir	virulence
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
X-gluc	5-bromo-4-chloro-indolyl-β-D-glucuronide
YEP	yeast peptone

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PREFACE

Coniothyrium zuluense is the causal agent of a serious *Eucalyptus* stem canker disease in South Africa (Wingfield *et al.,* 1997). *Eucalypts* are the most important hardwood plantations in the world, and in South Africa these hardwoods occupy approximately 1.5 million hectares of plantation area, an area that is soon to be increased by an additional 600 000 hectares. As exotics, *Eucalyptus* plantations are constantly exposed to infection by fungal pathogens such as *C. zuluense,* which by secreting cell-wall degrading enzymes contribute to the degradation of plant cell walls and subsequent reduction and in the quality of timber produced. This ultimately affects the South African paper, pulp and timber industries.

Selection of resistant clones through traditional breeding methods is the most common method currently employed in overcoming the problem of fungal infection. The genetic manipulation of *Eucalyptus* trees for enhanced resistance to fungal diseases is an alternative to the time-consuming and tedious approach of conventional breeding. The identification of several antifungal proteins, particularly polygalacturonase-inhibiting proteins (PGIPs) from various plant species including *Eucalyptus,* lead to the hypothesis that over-expression of these proteins could potentially reduce pathogen attack. However, prior to the expression of PGIPs in plants, isolation and molecular characterization of these genes are required. The aims of this study were therefore (1) to clone and characterize the complete *Eucalyptus grandis pgip* gene, (2) to transform *Nicotiana tabacum* (tobacco) plants with the *E. grandis pgip* gene and (3) to test for inhibition of *C. zuluense* PGs by PGIPs extracted from transgenic tobacco plants. This forms the first step towards the generation of *E. grandis* clones that are more disease tolerant.

A review of the role of fungal endopolygalacturonases and polygalacturonase-inhibitors in plant-pathogen interactions are presented in chapter 1. Strategies employed to isolate and characterize *pgip* genes from a range of plant species are highlighted and the importance of PGIPs in disease resistance is discussed. In chapter 2, the molecular cloning and characterization of the *E. grandis pgip* gene is discussed. The work presented in this chapter is a follow up on work previously conducted by Chimwamurombe (2001). Previously, a partial *Eucalyptus pgip* gene sequence was obtained with the use of degenerate oligonucleotide primers. In this study, the complete *Eucalyptus pgip* gene was obtained through the employment of genome walking strategies.

Transformation of *Nicotiana tabacum* cv LA Burley plants with the *Eucalyptus pgip* gene and the molecular characterization of transgenic tobacco plants is discussed in chapter 3. The transformation and expression of foreign genes in tobacco plants is a well-established protocol, making tobacco the most appropriate candidate plant for assessing the functionality of the plant transformation construct. The production of endopolygalacturonases from virulent *C. zuluense* isolates and the subsequent PGIP assays conducted to determine levels of PG inhibition are included in this chapter.

This thesis consists of three independent chapters representing studies on the molecular characterization of an *E. grandis pgip* gene and focusing on the potential for inhibition of PGs produced by *C. zuluense* by *Eucalyptus* PGIP extracted from transgenic tobacco plants. Repetition of certain aspects in the individual chapters has been unavoidable and the thesis is presented following a uniform style.