

## TABLE OF CONTENTS

	<b>Page</b>
TABLE OF CONTENTS	i
LIST OF TABLES	ii
LIST OF FIGURES	iii
LIST OF ABBREVIATIONS	v
INTRODUCTION	1
OBJECTIVE	3
LITERATURE REVIEW	4
MATERIALS AND METHODS	21
RESULTS	37
DISCUSSION	64
CONCLUSION	70
LITERATURE CITED	71
APPENDIX	79

## LIST OF TABLES

Table	Page
1 Properties of the different <i>Potyvirus</i> proteins	6
2 Examples of coat protein-mediated resistance to virus infection	18
3 Number of putative transgenic papaya lines derived from four transgene constructs	21
4 Primers used for genes amplification by PCR	24
5 List of primers used for amplification of T-DNA flanking sequence	33
6 Molecular analysis of transgenic papaya R <sub>0</sub> lines for the presence of <i>CP</i> and <i>Nlb</i> transgenes	43
7 Evaluation of PRSV resistance in transgenic papaya R <sub>0</sub> lines containing PRSV transgenes under glasshouse condition	47
8 Evaluation of PRSV resistance in transgenic papaya R <sub>1</sub> lines	58

### Appendix Table

1 Composition of bacterium culture medium	80
2 Preparation of BSA standard curve for measuring the protein concentrations	81
3 Solutions for preparing 12% resolving gel for Tris-glycine SDS-polyacrylamide gel electrophoresis	82
4 Solutions for preparing 5% stacking gels for Tris-glycine SDS-polyacrylamide gel electrophoresis	82

## LIST OF FIGURES

Figure	Page
1      Organization of <i>Potyvirus</i> genome	5
2      PRSV infected papaya developed various symptoms	8
3      Organization and expression of a <i>Potyvirus</i> genome (Polyprotein strategy)	10
4      Schematic structure of the <i>Agrobacterium</i> Ti-plasmid and orientation of T- DNA integration in plant DNA	12
5      Schematic representation of CP structure and the position of the conserved DAG motif	17
6      Schematic representation of the orientation of genes in the chimeric constructs for papaya transformation	22
7      Location of specific primers designed from the known sequences of construct	33
8      Analysis of genomic DNA extracted from young fresh leaves of papayas by agarose gel electrophoresis	38
9      PCR analysis of PRSV-derived transgenes in putative transgenic papaya lines	39
10     Synthesis of the DIG-labelled DNA probes using the DIG-11-dUTP-labelling mixture (Roche)	40
11     Analysis of number of transgene in transgenic papaya genome by Southern blot hybridization	42
12     Symptom observations of the inoculated transgenic papaya lines containing PRSV-derived transgenes after 14 days of the last inoculation	45
13     Detection of <i>NIb</i> and <i>CP</i> transgenes in the transgenic resistant lines by RT-PCR after inoculation	46
14     Analysis of the accumulation of CP transcripts in the uninoculated transgenic resistant lines by Northern blot hybridization	51

## LIST OF FIGURES (continued)

Figure	Page
15      Analysis of the accumulation of CP transcripts in the transgenic resistant lines during PRSV pre-inoculation and post-inoculation	53
16      Analysis of PRSV-CP in the uninoculated transgenic resistant lines by Western blot analysis	54
17      Analysis of the accumulation of NIb transcripts in the uninoculated transgenic resistant lines by Northern hybridization	55
18      Analysis of PCR product of amplified fragments derived from long PCR	60
19      Analysis of nucleotide sequence showed the location of the elements in plant expression construct	62
20      Sequence analysis of SL2 and pMON65301 using SeqMan program	63

## LIST OF ABBREVIATIONS

bp	=	Base pair
CDP- <i>Star</i>	=	Disodium-4-chloro-3-(methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro) tricycle[3.3.1.1. <sup>3,7</sup> ] decan}-4-yl)-1-phenyl phosphate
CTAB	=	Cethyl Trimethyl Ammonium Bromide
DIG-11-dUTP	=	Digoxigenin-11-uridine-5'-triphosphate
DNA	=	Deoxyrionucleic acid
dNTP	=	Deoxyrionucleotide triphosphate
EDTA	=	Ethylene diamine tetraacetic acid
g	=	Gram
h	=	Hour
kb	=	Kilobase
kDa	=	Kilodalton
µg	=	Microgram
µl	=	Microlitre
LB	=	Luria bertani broth
M	=	Molar
mM	=	Millimolar
mg	=	Milligram
ml	=	Millilitre
ng	=	Nanogram
<i>nptII</i>	=	Neomycin phosphotransferase II gene
OD	=	Optical density
ORF	=	Open reading frame
PBS	=	Phosphate buffer saline
PCR	=	Polymerase chain reaction
PRSV	=	<i>Papaya ringspot virus</i>
RNA	=	Ribonucleic acid
RT-PCR	=	Reverse transcription-Polymerase chain reaction
rpm	=	Round per minute

rRNA	=	Ribosomal RNA
SDS	=	Sodium dodecyl sulfate
SDS-PAGE	=	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SSC	=	Saline-sodium-citrate
TAE	=	Tris acetate EDTA
TEMED	=	N, N, N', N'-tetra methylethylene diamine
UV	=	Ultraviolet
V	=	Volts