

DIVERSITY OF VIRUSES
INFECTING *DIOSCOREA* SPECIES
IN THE SOUTH PACIFIC

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The University of Greenwich

Natural Resources Institute

2002

DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

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ABSTRACT

Yam (*Dioscorea* species) is a staple food crop in the South Pacific islands. Viruses infecting yams are poorly characterised, which is a hindrance to the safe movement of germplasm. Consequently, a comparison of Enzyme-linked immunosorbent assay (ELISA), Immunosorbent electron microscopy (ISEM), and Polymerase chain reaction (PCR) was carried out in order to identify the most reliable technique for the detection of yam viruses.

ELISA was used to examine the presence of *Yam mosaic virus* (YMV), *Dioscorea alata potyvirus* (DAV), *D. dumetorum potyvirus* (DDV), *D. alata badnavirus* (DABV), *D. bulbifera badnavirus* (DBBV), *Dioscorea latent potexvirus* (DLV), and *Cucumber mosaic virus* (CMV) in leaf samples from 719 plants representing nine *Dioscorea* species from seven South Pacific countries. DAV was the most prevalent virus in this region (69% of samples prevalent ELISA-positive).

The variability of DAV and *Badnavirus* was assessed for the development of sensitive diagnostic methods. DAV isolates were extracted from 37 samples representing three *Dioscorea* species from the South Pacific. *Dioscorea badnavirus* isolates were extracted from 37 samples representing seven *Dioscorea* species from the South Pacific and Africa. The phylogenetic analysis revealed that DAV isolates form a distinct group among potyviruses whereas *Dioscorea* badnaviruses cluster into a number of subgroups that were distinct from other badnaviruses.

In vitro culture was successfully carried out on 64 accessions including species of *D. alata*, *D. rotundata*, *D. dumetorum*, and *D. sansibarensis* from Africa and the South Pacific. DAV-specific RT-PCR results indicated that electrotherapy eliminated DAV from 62% of treated-DAV infected nodes. Chemotherapy and thermotherapy eliminated DAV from 7%, and 43% of node cutting respectively, while hot water therapy had no effect on 16 treated node cuttings.

These results are discussed in relation to the need to provide reliable virus indexing and virus elimination methods to facilitate the safe movement and exchange of yam germplasm within and outside the South Pacific region.

TABLE OF CONTENTS

AKNOWLEDGEMENTS	i
ABSTRACT	iii
LIST OF ABBREVIATIONS	xi
LIST OF TABLES	x
LIST OF FIGURES	xvi
CHAPTER 1	
INTRODUCTION	1
CHAPTER 2	
LITERATURE REVIEW.....	5
1- TAXONOMY AND MORPHOLOGY OF YAMS	5
2- ORIGIN AND DISTRIBUTION OF YAMS	9
3- CULTIVATION OF YAMS	12
4- YIELD LOSSES	12
4.1- NEMATODE, INSECT AND VERTEBRATE PESTS OF YAM	13
4.2- FUNGAL AND BACTERIAL DISEASES OF YAM	15
4.3- VIRAL DISEASES OF YAM	17
4.3.1- Family: Potyviridae.....	19
4.3.2- Family: Caulimoviridae, genus: Badnavirus.....	29
4.3.3- Family: Bromoviridae, genus: Cucumovirus.....	32
4.3.4- Unclassified yam viruses.....	34
5- DIAGNOSTIC TECHNIQUES	36
5.1- IDENTIFICATION OF VIRUSES INFECTING YAMS IN THE SOUTH PACIFIC ISLANDS	39
5.1.1- Enzyme-linked immunosorbent assay.....	39
5.1.2- Transmission electron microscopy.....	40
5.2- GENETIC VARIABILITY OF VIRUSES PRESENT IN YAMS FROM THE SOUTH PACIFIC	41
5.2.1- Polymerase chain reaction.....	41
5.2.2- Restriction fragment length polymorphism.....	44
5.2.3- Nucleotide sequencing.....	44
5.3- COMPARISON OF SEROLOGY AND PCR TECHNIQUES FOR VIRUS DETECTION.....	46
6- PLANT TISSUE CULTURES	46
6.1- CLONAL MULTIPLICATION.....	47
6.2- PRODUCTION OF VIRUS-FREE PLANTS.....	48

CHAPTER 3

MATERIALS AND METHODS.....	51
1- TEST PLANTS	51
1.1- LEAF SAMPLES	51
1.2- TUBER SAMPLES	51
2- ENZYME-LINKED IMMUNOSORBENT ASSAY.....	52
2.1- VIRUS EXTRACTION	52
2.2- ANTISERUM	53
2.3- TAS-ELISA	53
2.4- PAS-ELISA.....	55
2.5- DAS-ELISA.....	55
3- IMMUNOSORBENT ELECTRON MICROSCOPY.....	56
4- RT-PCR FOR THE DETECTION OF RNA VIRUSES	56
4.1- RNA EXTRACTION.....	56
4.2- REVERSE TRANSCRIPTION.....	57
4.3- POLYMERASE CHAIN REACTION	58
4.3.1- PCR procedure for the detection of <i>D. alata</i> and <i>D. dumetorum</i> potyviruses.....	58
4.3.2- PCR procedure for the detection of <i>Dioscorea</i> latent potyvirus.....	62
4.3.3- PCR procedure for the detection of Cucumber mosaic virus.....	62
5- PCR FOR THE DETECTION OF <i>DIOSCOREA</i> BADNAVIRUSES.....	63
5.1- DNA EXTRACTION.....	63
5.2- POLYMERASE CHAIN REACTION	64
5.2.1- Primers.....	64
5.2.2- PCR reaction.....	64
6- RESTRICTION FRAGMENT LENGTH POLYMORPHISM.....	66
7- CLONING.....	66
7.1- PURIFICATION OF DNA FRAGMENTS.....	66
7.2- LIGATION OF PLASMID VECTOR AND INSERT DNA	67
7.3- TRANSFORMATION OF DNA INTO BACTERIA AND TRANSFORMATION EFFICIENCY	67
7.3.1- Transformation.....	67
7.3.2- Transformation efficiency.....	68
7.4- PLASMID DNA PURIFICATION AND QUANTIFICATION.....	69
7.4.1- Plasmid DNA purification.....	69
7.4.2- Purified plasmid DNA quantification	70
8- SEQUENCING.....	70

CHAPTER 4

COMPARISON AND OPTIMISATION OF TECHNIQUES FOR THE DETECTION OF YAM VIRUSES71

1- INTRODUCTION.....71

2- MATERIALS AND METHODS72

2.1- VIRUS DISTRIBUTION DURING YAM GROWTH.....72

2.2- COMPARISON OF ELISA, ISEM AND PCR FOR THE DETECTION OF YAM VIRUSES ...72

2.2.1- *Detection of Dioscorea alata potyvirus*.....73

2.2.2- *Detection of Dioscorea dumetorum potyvirus*.....73

2.2.3- *Detection of Dioscorea latent potexvirus*.....73

2.2.4- *Detection of Dioscorea alata and D. bulbifera badnaviruses*.....73

2.2.5- *Detection of Cucumber mosaic virus*.....74

2.3- COMPARISON OF PAS-ELISA AND RT-PCR ON THE SAME LEAF74

2.4- PCR TECHNIQUE OPTIMISATION74

3- RESULTS76

3.1- VIRUS DISTRIBUTION DURING YAM GROWTH.....76

3.2- COMPARISON OF ELISA, ISEM AND PCR FOR THE DETECTION OF YAM VIRUSES ...78

3.2.1- *Detection of Dioscorea alata potyvirus*.....78

3.2.2- *Detection of Dioscorea dumetorum potyvirus*.....78

3.2.3- *Detection of Dioscorea latent potexvirus*.....78

3.2.4- *Detection of Dioscorea alata and D. bulbifera badnaviruses*.....80

3.2.5- *Detection of Cucumber mosaic virus*.....80

3.3- COMPARISON OF RT-PCR AND PAS-ELISA ON THE SAME LEAF80

3.4- PCR TECHNIQUE OPTIMISATION80

4- DISCUSSION83

4.1- LEAF SAMPLING FOR VIRUS INDEXING83

4.2- COMPARISON OF ELISA, ISEM AND PCR FOR THE DETECTION OF YAM VIRUSES ...85

4.2.1- *Factors affecting the methods of detection for yam viruses*.....85

4.2.2- *Methods of detection for each virus tested*.....87

4.3- COMPARISON OF ELISA AND RT-PCR ON THE SAME LEAF89

4.4- PCR OPTIMISATION89

4.5- CONCLUSIONS.....92

CHAPTER 5

VIRUS PREVALENCE IN THE SOUTH PACIFIC YAMS95

1- INTRODUCTION.....95

2- MATERIALS AND METHODS96

2.1- SAMPLES96

2.2- ELISA TEST96

2.2.1- *Antisera*96

2.2.2- *ELISA protocols*.....97

3- RESULTS97

3.1- LEAF SAMPLES97

3.2- ELISA TESTS.....98

3.3- ELISA DATA ANALYSIS.....99

4- DISCUSSION111

5- CONCLUSIONS117

CHAPTER 6

GENETIC VARIABILITY OF *DIOSCOREA* POTYVIRUSES INFECTING YAMS IN THE SOUTH PACIFIC ISLANDS120

1- INTRODUCTION.....	120
2- MATERIALS AND METHODS	121
2.1- PCR DETECTION	121
2.1.1- <i>Detection of Dioscorea dumetorum potyvirus</i>	121
2.1.2- <i>Detection of Dioscorea alata potyvirus</i>	122
2.2- CLONING AND SEQUENCING	124
3- RESULTS	126
3.1- PCR DETECTION	126
3.1.1- <i>Detection of Dioscorea dumetorum potyvirus</i>	126
3.1.2- <i>Detection of Dioscorea alata potyvirus</i>	126
3.2-SEQUENCE ANALYSIS OF DAV FROM THE CORE OF THE CP TO THE POLY (A) TAIL	130
3.3- SEQUENCE ANALYSIS OF DAV FROM THE NIB 3'-END TO THE CP 5'-END REGIONS	139
3.4- SEQUENCE ANALYSIS OF DAV CP FROM DAVCP01-F TO POT1CP-R PRIMERS	141
4- DISCUSSION	147
4.1- PCR DETECTION	147
4.1.1- <i>Detection of Dioscorea dumetorum potyvirus</i>	147
4.1.2- <i>Detection of Dioscorea alata potyvirus</i>	147
4.2- RFLP ANALYSIS	147
4.3- SEQUENCE ANALYSIS OF DAV FROM THE CORE OF THE CP TO THE POLY (A) TAIL	149
4.4- SEQUENCE ANALYSIS OF DAV FROM THE NIB 3'-END TO THE CP 5'-END REGIONS	151
4.5- SEQUENCE ANALYSIS OF DAV CP FROM DAVCP01-F TO POT1CP-R PRIMERS	152
5- CONCLUSIONS	153

CHAPTER 7

GENETIC DIVERSITY OF *DIOSCOREA* BADNAVIRUSES INFECTING YAMS IN THE SOUTH PACIFIC ISLANDS.....157

1- INTRODUCTION.....	157
2- MATERIALS AND METHODS	158
2.1- PCR DETECTION	158
2.2- CLONING AND SEQUENCING	160
3- RESULTS	160
3.1- PCR DETECTION	160
3.2- RFLP RESULTS	163
3.3- SEQUENCE ANALYSIS	164
4- DISCUSSION	174
4.1- PCR DETECTION	174
4.2- RFLP ANALYSIS	175
4.3- SEQUENCE ANALYSIS	176
5- CONCLUSIONS	180

CHAPTER 8

TISSUE CULTURE OF *DIOSCOREA* SPECIES182

1- INTRODUCTION.....182

2- MATERIALS AND METHODS184

2.1- CULTURE MEDIA PREPARATION184

2.1.1- Node cuttings184

2.1.2- Meristem-tip culture184

2.2- TOOLS AND SURFACE WORK PREPARATION185

2.3- SURFACE STERILISATION OF THE EXPLANTS185

2.3.1- Node cuttings185

2.3.2- Meristem-tip cultures.....186

2.4- CULTURING OF EXPLANTS187

2.4.1- Node cuttings187

2.4.2- Meristem-tip cultures.....187

2.5- *IN VITRO* CULTURE CONDITIONS.....187

2.6- ESTABLISHMENT OF PLANTLETS IN SOIL.....188

2.7- ESTABLISHMENT OF A YAM GERMPLASM COLLECTION.....188

2.8- TRIALS ON THE PRODUCTION OF VIRUS-FREE PLANTS.....189

2.8.1- Plant selection and preparation.....189

2.8.2- Nodes and meristems as material for starting explants.....189

2.8.3- Chemotherapy.....190

2.8.4- Thermotherapy.....192

2.8.5- Electrotherapy.....193

2.8.6- Hot water therapy193

2.8.7- Assessment of efficacy of therapies used for the production of DAV-free plants.....195

3- RESULTS196

3.1- ESTABLISHMENT OF A YAM GERMPLASM COLLECTION.....196

3.2- TRIALS ON THE PRODUCTION OF VIRUS-FREE PLANTS.....200

3.2.1- Comparison of nodes and meristems as material for starting explants.....200

3.2.2- Chemotherapy.....200

3.2.3- Thermotherapy.....201

3.2.4- Electrotherapy.....206

3.2.5- Hot water therapy210

3.2.6- Assessment of efficacy of therapies used for the production of DAV-free plants.....210

4- DISCUSSION213

4.1- CREATION OF AN *IN VITRO* YAM GERMPLASM COLLECTION.....213

4.2- TRIALS ON THE PRODUCTION OF VIRUS-FREE PLANTS.....215

5- CONCLUSIONS221

CHAPTER 9

GENERAL CONCLUSIONS AND FUTURE WORK223

REFERENCES230

ANNEX 1

DESCRIPTOR OF LEAF SAMPLES271

ANNEX 2

CTAB RNA EXTRACTION.....286

ANNEX 3

ELISA RESULTS287

ANNEX 4

ELECTROTHERAPY EXPERIMENT.....302

LIST OF ABBREVIATIONS

A	adenine
aa	amino acids
Abs	absorbance
AFLP	amplified fragment length polymorphism
AIP	alkaline phosphatase
AMV	<i>Avian myeloblastosis virus</i>
AMV	<i>Arabis mosaic virus</i>
BAP	6-benzyl-aminopurine
BBrMV	<i>Banana bract mosaic virus</i>
BBTV	<i>Banana bunchy-top virus</i>
BCMV	<i>Bean common mosaic virus</i>
bp	base pair(s)
BSV	<i>Banana streak virus</i>
BtMV	<i>Beet mosaic virus</i>
BYMV	<i>Bean yellow mosaic virus</i>
C	cytosine
°C	degree Celsius
CdMV	<i>Cardamom mosaic virus</i>
cDNA	complementary DNA, copy from RNA
CYNMV	<i>Chinese yam necrotic mosaic virus</i>
C ₂ H ₄ O ₂	glacial acetic acid
CI	cylindrical inclusion
cm	centimetre(s)
CMBV	<i>Citrus mosaic badnavirus</i>
CMV	<i>Cucumber mosaic virus</i>
CoYMV	<i>Commelina yellow mottle virus</i>
CP	coat protein
CSSV	<i>Cocoa swollen shoot virus</i>
CVC	clarified viral concentrate
CTAB	cetyltrimethylammonium bromide
CyMV	<i>Cymbidium mosaic potyvirus</i>
DABV	<i>Dioscorea alata badnavirus</i>
DARMV	<i>Dioscorea alata ring mottle virus</i>
DAS-ELISA	double antibody sandwich-ELISA
DAV	<i>Dioscorea alata potyvirus</i>
DBBV	<i>Dioscorea bulbifera badnavirus</i>
DDV	<i>Dioscorea dumetorum potyvirus</i>
DEP	dilution end point of the infectivity of sap
DEPC	diethylpyrocarbonate
DGBV	<i>Dioscorea green-banding virus</i>
DGBMV	<i>Dioscorea green-banding mosaic virus</i>
DIECA	dethyldithiocarbamate
DLV	<i>Dioscorea latent potyvirus</i>

DMCV	<i>Dioscorea mild chlorotic virus</i>
DMSO	dimethylsulphoxide
DMV	<i>Dioscorea mottle virus</i>
DNA	deoxyribonucleic acid
DNV	<i>Dioscorea necrotic virus</i>
dNTPs	deoxynucleotide triphosphate(s)
ddNTPs	dideoxynucleotide triphosphate(s)
DTV	<i>Dioscorea trifida potyvirus</i>
DTT	dithiothreitol
EDTA	ethylenediamine tetra acetic acid
EM	electron microscopy
ELISA	enzyme-linked immunosorbent assay
GA3	gibberellic acid
g	gram(s)
G	guanine
GVBAV	<i>Gooseberry vein banding associated virus</i>
h	hour(s)
HC	helper component
HCl	hydrochloric acid
IAA	isoamyl alcohol
IACR	Institute of Arable Crops Research (UK)
IBS	internal brown spot
IC-PCR	immunocapture-PCR
IC-RT-PCR	immunocapture-RT-PCR
IgG	immunoglobulin G
IITA	International Institute of Tropical Agriculture (Nigeria)
IMS	industrial methyl spirit
IPTG	isopropyl-thiogalactoside
ISEM	immunosorbent electron microscopy
JYMV	<i>Japanese yam mosaic virus</i>
6K1	first 6K peptide
6K2	second 6K peptide
kbp	kilobase pair(s)
KC ₂ H ₃ O ₂	potassium acetate
KCl	potassium chloride
kg	kilogram(s)
KH ₂ PO ₄	potassium phosphate (monobasic)
KC ₂ H ₃ O ₂	potassium acetate
l	litre(s)
LB	Luria-Bertani medium
LIV	longevity of the infectivity of sap <i>in vitro</i>
m	meter(s)
M	Molar(s)
M-IC-(RT)-PCR	multiplex-IC-(RT)-PCR
mA	milliampere(s) = 10 ⁻³ Ampere
MAbs	monoclonal antibodies
MacMV	<i>Maclura mosaic virus</i>
mg	milligram(s) = 10 ⁻³ gram

MgCl ₂	magnesium chloride
min	minute(s)
ml	millilitre(s) = 10 ⁻³ litre
mm	millimetre(s) = 10 ⁻³ metre
mm ²	square millimetre(s)
mM	millimolar(s) = 10 ⁻³ Molar
MP	mother-plant(s)
MS medium	Murashige and Skoog medium
NAA	naphtaleneacetic acid
NaCl	sodium chloride
Na ₂ CO ₃	sodium carbonate
NaHCO ₃	sodium hydrogen carbonate or sodium bicarbonate
Na ₂ HPO ₄	sodium phosphate (dibasic)
NaN ₃	sodium azide
NaOH	sodium hydroxide
Na ₂ SO ₃	sodium sulphite
NH ₄ C ₂ H ₃ O ₂	ammonium acetate
NiB	nuclear inclusion protein b
NLV	<i>Narcissus latent virus</i>
NRI	Natural Resources Institute
ng	nanogram(s) = 10 ⁻⁹ gram
nt(s)	nucleotide(s)
nm	nanometre(s) = 10 ⁻⁹ meter
ORF	open reading frames
ORSV	<i>Odontoglossum ringspot tobamovirus</i>
P1	first protein
P3	third protein
PAbs	polyclonal antibodies
PAS-ELISA	protein A sandwich-ELISA
PBS	phosphate buffer saline
PBS-T	phosphate buffer saline containing Tween 20
PCR	polymerase chain reaction
PEG	polyethylene glycol
PLRV	<i>Potato leaf roll virus</i>
PNP	nitrophenol phosphate
PNPP	nitrophenol diphosphate sodium
PPV	<i>Plum pox virus</i>
PVP-40	polyvinylpyrrolidone-40
PVS	<i>Potato virus S</i>
PVX	<i>Potato virus X</i>
PVY	<i>Potato virus Y</i>
PYMV	<i>Piper yellow mottle virus</i>
RFLP	restriction fragment length polymorphism
RNase H	Ribonuclease H
RNA	ribonucleic acid
RNase A	ribonuclease A
RNAsin	ribonucleic acid inhibitor
rpm	rotation per minute

RT	reverse transcriptase
RTBV	<i>Rice tungro bacilliform virus</i>
RT-PCR	Reverse transcription-polymerase chain reaction
s	second(s)
SbMV	<i>Soybean mosaic virus</i>
ScBV	<i>Sugarcane bacilliform virus</i>
ScMV	<i>Sugarcane mosaic virus</i>
SDS	sodium dodecyl sulphate
SDW	sterile distilled water
SPYN	South Pacific Yam Network
sp.	species (singular)
spp.	species (plural)
ssDNA	single-stranded DNA
ssRNA	single-stranded RNA
T	thymine
TAS-ELISA	triple antibodies sandwich-ELISA
TBE	tris borate- ethylenediamine tetra acetic acid
TBV	<i>Taro bacilliform virus</i>
TC	tissue culture
TIP	thermal inactivation point
T _m	melting temperature
TE	therapy efficiency
TEV	<i>Tobacco etch virus</i>
TMV	<i>Tomato mosaic virus</i>
Tris-HCl	Tris(hydroxymethyl)-aminomethane hydrochloric acid
TVBMV	<i>Tobacco vein-banding mosaic virus</i>
TVMV	<i>Tobacco vein mottling virus</i>
U	unit(s) of enzyme
µg	microgram(s) = 10 ⁻⁶ gram
µM	micromolar(s) = 10 ⁻⁶ Molar
µl	microlitre(s) = 10 ⁻⁶ litre
3'-UTR	3'-Untranslated region
UV	ultra violet
v	volume
V	volts
V _{pg}	genome-linked protein
w	weight
W	Watt(s)
WCIMV	<i>White clover mosaic potexvirus</i>
YMV	<i>Yam mosaic virus</i>
YMMV	<i>Yam mild mosaic virus</i> , also known as DAV
YVI	<i>Yam mosaic I</i> , also known as DAV
YV-N	<i>Nigerian yam virus</i>
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside

LIST OF TABLES

Table 1. Division of the genus <i>Dioscorea</i> according to morphological characteristics of the species (Alexander and Coursey, 1969)-----	7
Table 2. Common nematode, insect and vertebrate pests of yams -----	14
Table 3. Common fungal and bacterial diseases of yam -----	16
Table 4. Main characteristics of virus families infecting yam-----	18
Table 5. Members of the family Potyviridae transmitted by fungi, mites or whiteflies (Astier <i>et al.</i> , 2001a)-----	20
Table 6. Classification of yam viruses belonging to the family <i>Potyviridae</i> , according to the type of inclusion bodies (Walkey, 1991a)-----	20
Table 7. Functions of the different <i>Potyvirus</i> proteins (Urququi-Inchima <i>et al.</i> , 2001)-----	22
Table 8. Summary of characteristics of yam viruses belonging to the <i>Potyviridae</i> family -----	27
Table 8 (Continued). Summary of characteristics of yam viruses belonging to the <i>Potyviridae</i> family -	28
Table 9. Summary of characteristics of yam badnaviruses, potexviruses, and cucumoviruses -----	37
Table 9 (Continued). Summary of characteristics of yam badnaviruses, potexviruses, and cucumoviruses. -----	38
Table 10. Antisera used for the ELISA tests -----	54
Table 11. Description of primers used for the detection of DAV and DDV-----	59
Table 12. Description of primers used for the detection of <i>Dioscorea</i> badnaviruses -----	65
Table 13. Comparison of PAS-ELISA, ISEM, and RT-PCR for the detection of DAV-----	79
Table 14. Comparison of PAS-ELISA, ISEM, and RT-PCR for the detection of DDV-----	79
Table 15. Comparison of PAS-ELISA, ISEM, and PCR for the detection of DABV and DBBV -----	81
Table 16. Comparison of PAS-ELISA, ISEM, and RT-PCR for the detection of CMV -----	82
Table 17. Comparison of PAS-ELISA and RT-PCR for the detection of DAV from the same leaf -----	82
Table 18. Number of healthy <i>Dioscorea</i> species tested by ELISA-----	109
Table 19. Virus prevalence in plant species associated with yam cultivation in the South Pacific islands -----	110
Table 20. <i>Potyvirus</i> sequence references from the GenBank database-----	125
Table 21. RNAs extracted from different <i>Dioscorea</i> leaf samples and detected by RT-PCR using DAVCP01-F/Oligo d(T)-R primers-----	128
Table 22. RNAs extracted from different <i>Dioscorea</i> leaf samples, which were not detected by RT-PCR using DAVCP01-F/Oligo d(T)-R primers-----	129
Table 23. RFLP analysis of DAVCP01-F/Oligo d(T)-R PCR products using <i>TaqI</i> -----	132
Table 24. RFLP analysis of DAVCP01-F/Oligo d(T)-R PCR products from purified plasmid DNA using <i>TaqI</i> -----	133
Table 25. Sequence pair distance similarity of part of the core and the CP 3'-end and the 3'-UTR regions between DAV and other potyviruses -----	136
Table 26. RFLP analysis of YMMVS1-F/Pot1CP-R PCR products using <i>AluI</i> -----	140

Table 27. Sequence pair distance similarity of the NIb 3'-end and CP 5'-end regions between DAV and other potyviruses -----	143
Table 28. Sample numbers selected for the genetic variability studies -----	159
Table 29. <i>Badnavirus</i> sequence references from the GenBank database-----	162
Table 30. RFLP analysis of Badna-F/Badna-R PCR products using <i>AluI</i> -----	165
Table 31. RFLP analysis of Badna-F/Badna-R PCR products from purified plasmid DNA using <i>AluI</i> -	167
Table 32. RFLP analysis using <i>TaqI</i> of Badna-F/Badna-R PCR products from clones undigested with <i>AluI</i> -----	168
Table 33. Amino acid sequence pair distance similarity of part of the RT and RNase H regions between <i>Dioscorea</i> badnaviruses and other <i>Caulimoviridae</i> -----	172
Table 34. Chemicals used for the trial on the production of virus-free plants by chemotherapy-----	191
Table 35. Yam accessions from Africa transferred successfully to <i>in vitro</i> culture-----	197
Table 36. Yam accessions from the South Pacific islands transferred successfully to <i>in vitro</i> culture--	198
Table 37. Comparison of the success of the transfer in tissue culture of nodes and meristems from <i>D. alata</i> -----	202
Table 38. Chemotherapy efficiency for DAV elimination from node cuttings of <i>D. alata</i> PNG 189----	203
Table 39. Thermotherapy efficiency for DAV elimination from node cuttings from <i>D. alata</i> mother-plants transferred in to soil-----	204
Table 40. Thermotherapy efficiency for DAV elimination from node cuttings from <i>D. alata</i> mother-plants in tissue culture-----	205
Table 41. Effect of electrotherapy experiment on DAV elimination from node cuttings of <i>D. alata</i> PNG195 and on the maximal temperature reached -----	208
Table 42. Effect of electrotherapy experiment on DAV elimination from node cuttings of <i>D. alata</i> Van087 and on the maximal temperature reached -----	209
Table 43. Hot water therapy efficiency for DAV elimination from node cuttings of <i>D. alata</i> Van070 -	211
Table 44. Effect of chemotherapy, thermotherapy, electrotherapy and hot water therapy on the regeneration of <i>in vitro</i> <i>D. alata</i> plantlets from node cuttings and on DAV elimination -----	212

LIST OF FIGURES

Figure 1. Map showing the South Pacific islands, located north east of Australia-----	4
Figure 2. Morphology of six important cultivated yams (Barrau, 1956a)-----	6
Figure 3. Shapes of yam tubers; fusiform (top, from <i>D. alata</i>) and round (bottom, from <i>D. rotundata</i>)-	10
Figure 4. Origin and distribution of <i>Dioscorea</i> species (Degras, 1993d)-----	11
Figure 5. Organization of the <i>Potyvirus</i> genome -----	22
Figure 6. Mosaic leaf symptoms on <i>D. rotundata</i> infected with YMV (TDr87, right) compared to a healthy leaf from <i>D. rotundata</i> (DrGha2, left)-----	24
Figure 7. Mild mosaic leaf symptoms on <i>D. alata</i> infected with DAV (PNG189, right) compared to a healthy leaf from <i>D. alata</i> (PNG184, left) -----	25
Figure 8. DAV particles from <i>D. alata</i> collected in Vanuatu, observed by immunosorbent electron microscopy -----	25
Figure 9. Linear representation of <i>Badnavirus</i> genome showing the three ORFs (I, II and III), and the identified domains -----	31
Figure 10. Vein necrosis leaf symptoms on <i>D. esculenta</i> infected with DABV (PNG110, right) compared to a healthy leaf from <i>D. alata</i> (PNG184, left)-----	33
Figure 11. DABV extracted in <i>D. rotundata</i> collected from Ghana, observed by immunosorbent electron microscopy -----	33
Figure 12. Organization of the <i>Cucumvirus</i> genome -----	35
Figure 13. Organization of the <i>Potexvirus</i> genome -----	35
Figure 14. Virus distribution in plants of <i>D. rotundata</i> and <i>D. alata</i> infected with <i>Yam mosaic potyvirus</i> (YMV) and <i>D. alata potyvirus</i> (DAV) respectively -----	77
Figure 15. Prevalence of each virus in <i>D. alata</i> from the South Pacific islands detected by ELISA ----	102
Figure 15 (Continued). Prevalence of each virus in <i>D. alata</i> from the South Pacific islands detected by ELISA -----	103
Figure 16. Prevalence of CYNMV and JYMV in <i>D. alata</i> per Country detected by ELISA -----	104
Figure 17. Virus prevalence in <i>D. alata</i> from the South Pacific islands detected by ELISA.-----	105
Figure 18. Prevalence of each virus in <i>Dioscorea</i> species from the South Pacific islands detected by ELISA.-----	106
Figure 18 (Continued). Prevalence of each virus in <i>Dioscorea</i> species from the South Pacific islands detected by ELISA. -----	107
Figure 19. Prevalence of CYNMV and JYMV per <i>Dioscorea</i> species detected by ELISA. -----	108
Figure 20. Approximate position of primer pairs used for the detection of potyviruses -----	123
Figure 21. Restriction pattern of DAVCP01-F/Oligo d(T)-R PCR products using <i>TaqI</i> -----	131
Figure 22. Amino acid sequence alignment of part of the core and the C-terminal of CP of DAV -----	135
Figure 23. Phylogram from the amino acid alignment of part of CP region of DAV-----	137
Figure 24. Phylogram from the nucleotide alignment of the CP 3'-end and the 3'-UTR regions of DAV -----	138
Figure 25. Amino acid sequence alignment of the N1b 3'-end and the CP 5'-end regions of DAV -----	142

Figure 26. Phylogram from the amino acid alignment of the Nib 3'-end and CP 5'-end regions of DAV	144

Figure 27. Amino acid sequence alignment of DAV isolates from DAVCP01-F to Pot1CP-R primer -	145
Figure 28. Nucleotide sequence alignment of DAV isolates from the DAVCP01-F primer to Pot1CP-R primer-----	146
Figure 29. Approximate position of primer pairs used for the detection of badnaviruses -----	161
Figure 30. Restriction pattern of Badna-F/ Badna-R products from two to five clones from a same sample using <i>AluI</i> -----	166
Figure 31. Amino acid sequence alignment of part of the RT and beginning of RNase H regions of <i>Dioscorea</i> badnaviruses-----	170
Figure 31 (Continued). Amino acid sequence alignment of part of the RT and beginning of RNase H regions of <i>Dioscorea</i> badnaviruses -----	171
Figure 32. Phylogram from the amino acid alignment of part of the RT and RNase H regions of <i>Dioscorea</i> badnaviruses-----	173
Figure 33. Electrotherapy experiment -----	194
Figure 34. Tissue culture plantlets growing on node cutting media -----	199
Figure 35. Plantlets growing on soil one month after transfer-----	199
Figure 36. Temperature and power changes of PNG195 <i>D. alata</i> stem during the electrotherapy experiment -----	207