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

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A thesis submitted in partial fulfilment of the requirements of the University of Greenwich for the Degree of Doctor of Philosophy (PhD)

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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ACKNOWLEDGEMENTS

I would like to thank the Higher Education Funding Council for funding this project. My thanks to Dr V. Lebot, the co-ordinator of the South Pacific Yam Network (SPYN) project, who looked after me during my visit to Vanuatu. I express my sincere thanks to my supervisors, Dr L. Kenyon and Dr S.E. Seal, who gave me the great opportunity to do this PhD and for the time they spent advising and helping me.

Thank you to Dr J. Hughes, for welcoming me to the International Institute of Tropical Agriculture (IITA, Nigeria), and to all the people working in the virology laboratory there, and Dr S.Y.G. Ng for her advice on tissue culture. Also, I would like to thank Dr M. Taylor for use of the laboratory facilities of the Secretariat of the Pacific Community (Fiji), and E. Lesione for helping me in the tissue culture work there.

I would also like to thank Dr G. Jackson for his help in the project and all the following people in the South Pacific islands for collecting samples and sending them to NRI: Godwin Ala (Vanuatu); Moti Lala Autar (Fiji); Jimmy Risimeri (Papua New Guinea); Jimi Saelea (Solomon Islands); Didier Varin (New Caledonia).

I wish to acknowledge Dr J. Hughes and Dr N. Spence for kindly providing the antisera, Dr R.A. Mumford for supplying *Potexvirus* primers, and Dr G. Thottappilly and Dr G.J. Hafner for providing *Dioscorea badnavirus* primers. Thank you also to Dr R.A.A. Van Der Vlugt, for his help with the *Potexvirus* work. Acknowledgements to the people working in the electron microscopy unit, in particular to J. Devonshire and Dr P. Jones at the Institute of Arable Crops Research (IACR), Rothamsted, UK.

I am grateful to Dr E. Canning who trained me in molecular techniques, and for being patient with me. I greatly appreciate the laboratory support of M. Brown, and all the

technical staff, and other NRI staff who were always pleased to help. Although it is never easy to settle down in a new place, the NRI was a great place to meet people and to share the spirit of being a PhD student! Thanks to Henry, Cristina, Tiziana, Valerie, Delphine, Jeroen, Maruthi, Jamie, Yasin, Matt and others.

I would like to express my gratitude to my parents, for their moral support and financial help, and also to my sister and brother who came to visit me in England. Finally, to my beloved Arnaud who has always had the humour to cheer me up!

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.

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LIST OF ABBREVIATIONS

A	adenine
aa	amino acids
Abs	absorbance
AFLP	amplified fragment length polymorphism
AlP	alkaline phosphatase
AMV	*Avian myeloblastosis virus*
AMV	*Arabis mosaic virus*
BAP	6-benzyl-aminopurine
BBrMV	*Banana bract mosaic virus*
BBTV	*Banana bunchy-top virus*
BCMV	*Bean common mosaic virus*
bp	base pair(s)
BSV	*Banana streak virus*
BtMV	*Beet mosaic virus*
BYMV	*Bean yellow mosaic virus*
C	cytosine
^{o}C	degree Celsius
CdMV	*Cardamom mosaic virus*
cDNA	complementary DNA, copy from RNA
CYNMV	*Chinese yam necrotic mosaic virus*
$C_2H_4O_2$	glacial acetic acid
CI	cylindrical inclusion
cm	centimetre(s)
CMBV	*Citrus mosaic badnavirus*
CMV	*Cucumber mosaic virus*
CoYMV	*Commelina yellow mottle virus*
CP	coat protein
CSSV	*Cocoa swollen shoot virus*
CVC	clarified viral concentrate
CTAB	cetyltrimethylammonium bromide
CyMV	*Cymbidium mosaic potexvirus*
DABV	*Dioscorea alata badnavirus*
DARMV	*Dioscorea alata ring mottle virus*
DAS-ELISA	double antibody sandwich-ELISA
DAV	*Dioscorea alata potyvirus*
DBBV	*Dioscorea bulbifera badnavirus*
DDV	*Dioscorea dumetorum potyvirus*
DEP	dilution end point of the infectivity of sap
DEPC	diethylpyrocarbonate
DGBV	*Dioscorea green-banding virus*
DGBMV	*Dioscorea green-banding mosaic virus*
DIECA	dethyldithiocarbamate
DLV	*Dioscorea latent potexvirus*

DMCV	*Dioscorea mild chlorotic virus*
DMSO	dimethylsulphoxide
DMV	*Dioscorea mottle virus*
DNA	deoxyribonucleic acid
DNV	*Dioscorea necrotic virus*
dNTPs	deoxynucleotide triphosphate(s)
ddNTPs	dideoxynucleotide triphosphate(s)
DTV	*Dioscorea trifida potyvirus*
DTT	dithiothreitol
EDTA	ethylenediamine tetra acetic acid
EM	electron microscopy
ELISA	enzyme-linked immunosorbent assay
GA3	gibberellic acid
g	gram(s)
G	guanine
GVBAV	*Gooseberry vein banding associated virus*
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	*Japanese yam mosaic virus*
6K1	first 6K peptide
6K2	second 6K peptide
kbp	kilobase pair(s)
$KC_2H_3O_2$	potassium acetate
KCl	potassium chloride
kg	kilogram(s)
KH_2PO_4	potassium phosphate (monobasic)
$KC_2H_3O_2$	potassium acetate
l	litre(s)
LB	Luria-Bertani medium
LIV	longevity of the infectivity of sap *in vitro*
m	meter(s)
M	Molar(s)
M-IC-(RT)-PCR	multiplex-IC-(RT)-PCR
mA	milliampere(s) = 10^{-3} Ampere
MAbs	monoclonal antibodies
MacMV	*Maclura mosaic virus*
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_2CO_3	sodium carbonate
$NaHCO_3$	sodium hydrogen carbonate or sodium bicarbonate
Na_2HPO_4	sodium phosphate (dibasic)
NaN_3	sodium azide
NaOH	sodium hydroxide
Na_2SO_3	sodium sulphite
$NH_4C_2H_3O_2$	ammonium acetate
NIb	nuclear inclusion protein b
NLV	*Narcissus latent virus*
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	*Odontoglossum ringspot tobamovirus*
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	*Potato leaf roll virus*
PNP	nitrophenol phosphate
PNPP	nitrophenol diphosphate sodium
PPV	*Plum pox virus*
PVP-40	polyvinylpyrrolidone-40
PVS	*Potato virus S*
PVX	*Potato virus X*
PVY	*Potato virus Y*
PYMV	*Piper yellow mottle virus*
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	*Rice tungro bacilliform virus*
RT-PCR	Reverse transcription-polymerase chain reaction
s	second(s)
SbMV	*Soybean mosaic virus*
ScBV	*Sugarcane bacilliform virus*
ScMV	*Sugarcane mosaic virus*
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	*Taro bacilliform virus*
TC	tissue culture
TIP	thermal inactivation point
Tm	melting temperature
TE	therapy efficiency
TEV	*Tobacco etch virus*
TMV	*Tomato mosaic virus*
Tris-HCl	Tris(hydroxymethyl)-aminomethane hydrochloric acid
TVBMV	*Tobacco vein-banding mosaic virus*
TVMV	*Tobacco vein mottling virus*
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
Vpg	genome-linked protein
w	weight
W	Watt(s)
WClMV	*White clover mosaic potexvirus*
YMV	*Yam mosaic virus*
YMMV	*Yam mild mosaic virus*, also known as DAV
YVI	*Yam mosaic I*, also known as DAV
YV-N	*Nigerian yam virus*
X-Gal	5-bromo-4-chloro-3-indolyl-ß-D-galactoside

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