

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**CHARACTERISATION AND DETECTION OF
DASHEEN MOSAIC POTYVIRUS IN
*ZANTEDESCHIA***

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Horticultural Science in Plant Health at Massey University,
Palmerston North, New Zealand.

CHRISTINE GRACE MATTHEWS

1995

MASSEY UNIVERSITY



1061344802

ERRATA

Page 11	line 21: "a period of three months with which" should read " a period of three months in which"
Page 14	line 14: "Hendon" should read "Herndon" line 23: "Shawl" should read "Shaw". line 25: "Shukla and Ward, 1989" should read "Shukla and Ward, 1989a".
Page 15	Table 1.1: The first protein should be "P1" not "P3"
Page 20	line 1: "wide geographical" should read "widely separated geographical"
Page 22	line 22: "Dichlorovos®" should read "dichlorvos"
Page 39	line 7: "at 160,800g" should read "at 160,800g for 90 minutes"
Page 40	line 16: "200ul" should read "200ml".
Page 58	Table 3.1, column four, bottom line: "stuntimg" should read "stunting"
Page 67	line 3: "using two virus other isolates" should read "using two other virus isolates".
Page 73	line 18: "Enrlich" should read "Ehrlich"
Page 83	line 10: "feathery mottle potyvirus" should read "taro feathery mottle potyvirus".
Page 84	line 2: "numerable" should read "numerous" line 11: "not unlikely" should read "likely" line 13: "other" should read "some other" line 19: "would clarify whether" should read "would help confirm" line 19: "virus" should read "potyvirus"
Page 113	line 1: should read "Until this study no other potyvirus except DsMV has been ..."
Page 116	line 4: "infection should read "virus infection".
Page 126	Addition to the bibliography: Saiki (1989). The design and optimization of the PCR. In: PCR Technology. Ed. Ehrlich, G.D. M. Stockton Press. p7-16.

ABSTRACT

Four potyvirus isolates believed to be dasheen mosaic potyvirus, the most frequently occurring virus to infect members of the *Araceae*, were obtained from *Caladium*, *Colocasia*, *Xanthosoma* and *Zantedeschia* in world-wide locations. Properties of these isolates such as particle length, serological relatedness, electrophoretic mobility of coat proteins and genomic characteristics were compared.

Serologically distinct strains of dasheen mosaic potyvirus were apparent amongst the isolates. The difference in the serological relationship was coupled with a variation in symptom expression. An isolate from *Colocasia esculenta* (L.) Schott was not serologically related to the other isolates. Further isolates from *C. esculenta* also exhibited no relationship. The modal length was different as well as the ability of complementary deoxyribonucleic acid, produced to the viral ribonucleic acid, to bind with some of the primers used in the polymerase chain reaction. This evidence led to the proposal that the isolate from *C. esculenta* was not dasheen mosaic potyvirus; this virus is tentatively named taro feathery mottle potyvirus.

Cytoplasmic inclusion protein aggregates of dasheen mosaic potyvirus were purified from infected leaf tissue. SDS-polyacrylamide gel electrophoretic analysis of samples revealed a major band with an estimated molecular weight of 68,000 daltons. Such a band was absent from healthy tissue samples.

The ATPase activity in samples from each purification step was determined by measuring the amount of [32 P] released from the [γ - 32 P]ATP during incubation with the cytoplasmic inclusion protein. The level of ATPase activity in each sample showed a strong correlation with the amount of protein that was present.

In a limited survey of commercial plantings twenty nine tubers grown for cutflower or tuber export were obtained from seven properties at different locations in New Zealand and grown on in a greenhouse. Each plant was indexed for virus infection.

Electron microscopy revealed that plants from three of the properties contained 720nm flexuous rods. Samples from all but two plants tested positive to a potyvirus group antiserum using the enzyme-linked immunosorbent assay. The remaining two plants tested positive in microprecipitin and rapid immune electron microscopy tests to an antiserum prepared to a member of the carlavirus group. Particles from these plants were mechanically transmitted to *Nicotiana tabacum* 'Havana'.

Rod-shaped particles of 300nm were observed in plants from four properties and tested positive to tobacco mosaic tobamovirus antiserum using a microprecipitin test. While inoculations to herbaceous indicators resulted in no symptoms, 300nm particles were observed in samples from the indicator plants.

Tomato spotted wilt tospovirus, potato X potexvirus and cucumber mosaic cucumovirus, reported to infect *Zantedeschia* spp, were not detected.

ACKNOWLEDGEMENTS

I wish to thank my supervisors; Professor K.S. Milne for his support and encouragement, Dr R.L.S. Forster for his inspiration and enthusiasm that kept me going in the later stages of this study and Mr H.F. Neilson for his technical assistance and attempts to answer my numerous questions.

Thanks are also due to Doug Hopcroft and Raymond Bennett of the Electron Microscope Unit, Horticulture and Food Research Institute of New Zealand, Palmerston North for their assistance. It was a pleasure to work in such a well run laboratory.

The advice of Eddie Welsh and Alison Duffy on the culture of *Zantedeschia* was of great value. Mrs K. Hill also assisted by supplying *Zantedeschia* sp seedlings and advised on their care.

George Ionas and Carolyn Young, Department of Microbiology and Genetics, Massey University, willingly advised on some of the molecular biology techniques used. George Ionas' help with the PCR technique was of significant value and his support and encouragement was greatly appreciated.

Assistance was also given by Robin Eagles, Dave Beck and Davin Voot, Horticulture and Food Research Institute of New Zealand, Auckland. Their willingness to teach me some of the laboratory techniques used in this study was appreciated.

Thanks are due to Dr W. Zettler and Dr R. Fullerton for the supply of virus isolates.

I would also like to thank the staff of the Small Animal Production Unit, the Plant Growth Unit and the Central Photographic Unit at Massey University for their help.

Financial assistance was provided by the Leonard Condell Farming Trust Scholarship, Helen E. Akers Scholarship, Massey University Research Fund, Massey University Research Awards, and the NZ Nurserymen's Association Incorporated. This study could not have taken place without such assistance.

The friendship and encouragement of fellow postgraduates in the department was appreciated.

Finally, my greatest debt belongs to my family. Thank you to my husband Murray who has been willing to assist at home and has given the support necessary to ensure I completed this thesis. My children Janetta and Timothy have been very understanding as I have attempted to juggle my study and family commitments.

TABLE OF CONTENTS

TITLE	i
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xii

CHAPTER ONE: INTRODUCTION 1

1.1.	The family <i>Araceae</i>	5
1.2.	The genus <i>Zantedeschia</i>	5
1.3.	Virus infection of callas.	7
1.3.1.	Dasheen mosaic potyvirus.	7
1.3.2.	Tomato spotted wilt tospovirus.	10
1.3.3.	Cucumber mosaic cucumovirus.	10
1.3.4.	Potato X potexvirus and tobacco mosaic tobamovirus.	11
1.4.	Detection of dasheen mosaic potyvirus.	11
1.5.	The potyviruses.	13
1.6.	The polymerase chain reaction.	17
1.7.	Aims of this study.	19

CHAPTER TWO: A COMPARATIVE STUDY OF FOUR ISOLATES OF DASHEEN MOSAIC POTYVIRUS - MATERIALS AND METHODS .. 21

2.1.	Plant material.	21
2.2.	Host range studies.	23
2.3.	Electron microscopy.	24
2.3.1.	Direct investigation of leaf tissue.	24
2.3.2.	Investigation of purified viral preparations.	28
2.3.3.	Determination of viral particle length.	28

2.4.	Serology.	29
2.4.1.	Antiserum production.	29
2.4.2.	Enzyme-linked immunosorbent assay (ELISA).	30
2.4.2.1.	Antiserum purification for ELISA tests. .	30
2.4.2.2.	Enzyme conjugation of purified gamma-globulins	31
2.4.2.3.	ELISA methods.	32
2.4.2.4.	Optimisation of levels of ELISA reactants.	34
2.4.2.5.	Homologous and heterologous reactions of each isolate to individual antisera. ...	34
2.5.	Electrophoresis.	35
2.5.1.	SDS-polyacrylamide gel electrophoresis.	35
2.5.2.	Preparation of samples for SDS-PAGE.	36
2.5.3.	Non-denaturing electrophoresis of nucleic acids.	36
2.6.	Purification of viruses.	37
2.6.1.	Purification of dasheen mosaic potyvirus.	37
2.6.2.	Purification of cucumber mosaic cucumovirus.	45
2.7.	RNA extraction.	46
2.8.	Production of first strand cDNA.	48
2.9.	The polymerase chain reaction.	50
2.10.	Extraction of DNA from agarose gels.	52
2.10.1.	Extraction of DNA from standard agarose.	52
2.10.1.	Extraction of DNA from low melting point agarose. .	53
2.11.	Restriction endonuclease digestion.	54
2.12.	Cloning of PCR products.	54
2.12.1.	Ligation.	54
2.12.2.	Transformation.	55
2.12.3.	One tube plasmid DNA miniprep.	55

CHAPTER THREE: A COMPARATIVE STUDY OF FOUR ISOLATES OF
DASHEEN MOSAIC POTYVIRUS - RESULTS AND DISCUSSION. . . 57

3.1.	Host range studies.	57
3.2.	Electron microscopy.	61
3.3.	Serology.	64
3.4.	SDS-polyacrylamide gel electrophoresis.	69
3.5.	Genomic characteristics.	71
3.5.1.	Isolation of RNA.	71
3.5.2.	Synthesis of first strand cDNA.	71
3.5.3.	The polymerase chain reaction.	72
3.5.3.1.	Primers U335 and D335.	73
3.5.3.2.	Primers U335 and RLFS-9.	79
3.5.3.3.	Primers JF3 and D335 or RLFS-9.	79
3.5.4.	Cloning and sequencing of PCR products.	81
3.6.	Discussion.	82

CHAPTER FOUR: CYTOPLASMIC INCLUSION PROTEINS OF DASHEEN
MOSAIC POTYVIRUS. 85

4.1.	Introduction.	85
4.2.	Materials and Methods.	86
4.2.2.	Plant material and virus isolates.	86
4.2.2.	CI protein purification.	86
4.2.3.	Protein determination.	87
4.2.4.	Gel electrophoresis.	88
4.2.5.	ATPase activity assay.	89
4.3.	Results and discussion.	89

CHAPTER FIVE: SURVEY	97
5.1. Introduction.	97
5.2. Survey method.	97
5.3. Electron microscopy.	98
5.3.1. Direct investigation of leaf tissue.	98
5.3.2. Rapid immune electron microscopy.	98
5.4. Enzyme-linked immunosorbent assay.	99
5.4.1. Double antibody sandwich ELISA (DAS-ELISA). ...	99
5.4.2. Indirect ELISA.	99
5.5. Isolation of double-stranded RNA (dsRNA).	100
5.6. Mechanical inoculation to herbaceous indicators.	102
5.7. Results.	103
5.7.1. Electron microscopy.	105
5.7.2. Enzyme-linked immunosorbent assay.	107
5.7.3. dsRNA extraction.	108
5.7.4. Mechanical inoculation.	108
5.8. Discussion.	110
CHAPTER SIX: DISCUSSION	113
BIBLIOGRAPHY	117
APPENDIX ONE	131
APPENDIX TWO	132

LIST OF TABLES

Table 1.1.	Potyvirus proteins and their function	15
Table 2.1.	Concentration of coating gamma-globulins and dilutions of conjugated gamma-globulins of dasheen mosaic potyvirus (DsMV) antisera used in ELISA studies.	35
Table 2.2.	Comparison of various methods used to purify dasheen mosaic potyvirus (DsMV).	44
Table 2.3.	Sequences of oligonucleotide primers used in the polymerase chain reaction (PCR).	51
Table 3.1.	Symptoms of infection in various <i>Araceae</i> associated with four isolates of dasheen mosaic potyvirus.	58
Table 3.2.	Molecular weight estimates of SDS-degraded coat proteins of four isolates of dasheen mosaic potyvirus (DsMV) by electrophoresis in a SDS-polyacrylamide gel.	70
Table 5.1.	Indicator plants used in mechanical inoculation tests of survey material for detection of viruses in <i>Zantedeschia</i> spp.	103
Table 5.2.	Results of a survey to detect viruses in commercial plantings of <i>Zantedeschia</i> spp.	104

LIST OF FIGURES

Figure 1.1.	Mosaic symptoms on the leaves of <i>Zantedeschia</i> sp 'Tinkerbelle'.	2
Figure 1.2.	Distorted leaf of <i>Zantedeschia</i> sp 'Pink Persuasion'.	2
Figure 1.3.	Reduced plant growth of <i>Zantedeschia</i> sp 'Pink Persuasion'.	3
Figure 1.4.	Stunted plant of <i>Zantedeschia</i> sp 'Majestic Red'.	3
Figure 1.5.	Distorted spathe of <i>Zantedeschia</i> sp 'Majestic Red'.	4
Figure 1.6.	Colour break on the spathe of <i>Zantedeschia</i> sp 'Pink Persuasion'.	4
Figure 1.7.	Genome map of potyviruses.	15
Figure 2.1.	Leaf dip of DsMV infected <i>Caladium candidum</i> tissue in 2% potassium phosphotungstic acid (PTA), pH 7.0: bactracin (300ug/ml) (7:1).	25
Figure 2.2.	Leaf dip of DsMV infected <i>Caladium candidum</i> tissue in 2% potassium phosphotungstic acid (PTA) pH 7.0: 2% uranyl acetate (UA) pH 4.0 (1:1).	26
Figure 2.3.	Leaf dip of DsMV infected <i>Caladium candidum</i> tissue in 2% potassium phosphotungstic acid (PTA) pH 7.0: 2% ammonium molybdate (AM) pH 5.6 (2:1).	26
Figure 2.4.	Leaf dip of DsMV infected <i>Caladium candidum</i> tissue in 2% potassium phosphotungstic acid (PTA) pH 7.0: 2% ammonium molybdate (AM) pH 5.6 (1:1).	27
Figure 2.5.	Leaf dip of DsMV infected <i>Caladium candidum</i> tissue in 2% potassium phosphotungstic acid (PTA) pH 7.0: 2% ammonium molybdate (AM) pH 5.6 (1:2).	27
Figure 2.6.	Optical density profile of sucrose density gradients made up in 20mM Tris and 10mM EDTA of a preparation of DsMV purified by a modification of Abo El-Nil et al (1977).	41

Figure 2.7.	Optical density profile of sucrose density gradients made up in 0.005M borate and 0.2% SSC of a preparation of DsMV purified by a modification of Abo El-Nil et al (1977).	41
Figure 2.8.	Partially purified preparation of DsMV, after resuspension of a polyethylene glycol 6000 (PEG) precipitate, showing lateral aggregation.	43
Figure 3.1.	<i>Xanthosoma</i> sp inoculated with DsMV-Z showing severe stunting of emergent leaves.	59
Figure 3.2.	<i>Xanthosoma</i> sp infected with DsMV showing severe stunting.	59
Figure 3.3.	<i>Zantedeschia</i> sp 'Pink Persuasion' inoculated with DsMV-X showing distinct yellow mosaic symptoms on leaves.	60
Figure 3.4.	<i>Zantedeschia</i> sp 'Pink Persuasion' inoculated with DsMV-H showing necrotic leaf margins and reduced growth.	60
Figure 3.5.	Particle length distribution of dasheen mosaic potyvirus isolate DsMV-FL in leaf sap.	62
Figure 3.6.	Particle length distribution of dasheen mosaic potyvirus isolate DsMV-H in leaf sap.	62
Figure 3.7.	Particle length distribution of dasheen mosaic potyvirus isolate DsMV-X in leaf sap.	63
Figure 3.8.	Particle length distribution of dasheen mosaic potyvirus isolate of DsMV-Z in leaf sap.	63
Figure 3.9.	Absorbance of a dilution series of four isolates of dasheen mosaic potyvirus (DsMV) in leaf sap with a homologous/heterologous ELISA test using antiserum to the DsMV-FL isolate.	65

Figure 3.10.	Absorbance of a dilution series of four isolates of dasheen mosaic potyvirus (DsMV) in leaf sap with a homologous/heterologous ELISA test using antiserum to the DsMV-Z isolate.	65
Figure 3.11.	Absorbance of a dilution series of four isolates of dasheen mosaic potyvirus (DsMV) in leaf sap with a homologous/heterologous ELISA test using antiserum to the DsMV-X isolate.	66
Figure 3.12.	Absorbance of a dilution series of four isolates of dasheen mosaic potyvirus (DsMV) in leaf sap with a homologous/heterologous ELISA test using antiserum to the DsMV-H isolate.	66
Figure 3.13.	Absorbance of a dilution series of three dasheen mosaic potyvirus (DsMV)-like isolates from <i>Colocasia esculenta</i> leaf sap and tamarillo mosaic potyvirus (TaMV) in <i>Nicotiana benthamiana</i> leaf sap with an ELISA test using antiserum to the DsMV-H isolate of DsMV.	68
Figure 3.14.	Absorbance of a dilution series of four isolates of dasheen mosaic potyvirus (DsMV) and a DsMV-like isolate from <i>Colocasia esculenta</i> in leaf sap using antiserum to the potyvirus group.	68
Figure 3.15.	SDS-PAGE analysis of degraded coat proteins of four isolates of dasheen mosaic potyvirus (DsMV).	70
Figure 3.16.	ssRNA isolated from purified viral preparations of four isolates of dasheen mosaic potyvirus.	72
Figure 3.17(a).	Amplification of cDNA of DsMV-Z using the primers D335 and U335, and an annealing temperature of 41°C	75
Figure 3.17(b).	Amplification of cDNA of DsMV-Z using the primers D335 and U335, and an annealing temperature of 45°C	75

Figure 3.18.	Reamplification of PCR products using the primers U335 and D335	77
Figure 3.19.	Proposed binding sites of the primers U335 and D335 to cDNA from DsMV RNA.	78
Figure 3.20.	Amplification of cDNA of four isolates of DsMV using the primers U335 and RLFS-9.	80
Figure 3.21.	Amplification of cDNA of DsMV using the primers JF3 and D335.	80
Figure 3.22.	Proposed serological relationship between four isolates of dasheen mosaic potyvirus.	83
Figure 4.1.	SDS-PAGE analysis of samples from healthy and DsMV-infected <i>Zantedeschia</i> sp leaf tissue collected from each step during purification of CI proteins.	90
Figure 4.2.	Protein concentration and ATPase activity in samples collected from each step during purification of CI proteins from DsMV-FL infected <i>Caladium candidum</i> leaf tissue.	91
Figure 4.3.	Protein concentration and ATPase activity in samples collected from each step during purification of CI proteins from DsMV-X infected <i>Xanthosoma</i> sp tissue	91
Figure 4.4.	Protein concentration and ATPase activity in samples collected from each step during purification of CI proteins from DsMV-Z infected <i>Zantedeschia</i> sp leaf tissue	92
Figure 4.5.	Protein concentration and ATPase activity in samples collected from sucrose gradient fractions during purification of CI proteins from DsMV-X infected <i>Xanthosoma</i> sp leaf tissue.	94

Figure 4.6.	SDS-PAGE analysis of samples collected from sucrose gradient fractions during purification of CI proteins from DsMV-X infected <i>Xanthosoma</i> sp leaf tissue	94
Figure 5.1.	Leaf dip of tissue from a <i>Zantedeschia</i> sp grown for commercial production in the Bay of Plenty showing particles of similar morphology to tobacco mosaic tobamovirus	106
Figure 5.2.	Particle from <i>Zantedeschia</i> sp leaf tissue coated with TMV antiserum.	106
Figure 5.3.	Particle from <i>Zantedeschia</i> sp seed coat.	107
Figure 5.4.	Leaf dip of tissue from <i>Nicotiana tabacum</i> 'Havana' inoculated with leaf sap from a <i>Zantedeschia</i> sp grown for commercial production at a Horowhenua property	109
Figure 5.5.	Leaf tissue extracts from a <i>Zantedeschia</i> sp grown for commercial production at a Horowhenua property, treated according to the decoration technique of Milne and Luisoni (1977) using antiserum to carnation latent carlavirus.	110