Characterisation of Hardenbergia mosaic virus and development of microarrays for detecting viruses in plants

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by

Craig Graham Webster

B.Sc. Biotechnology and Chemistry

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State Agricultural Biotechnology Centre

School of Biological Sciences and Biotechnology

Murdoch University

Perth, Western Australia

Declaration

I declare that this is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institute.

Craig Graham Webster

Abstract

A virus causing chlorosis and leaf distortion in the Western Australian endemic legume *Hardenbergia comptoniana* was detected by biological indexing to *Chenopodium quinoa* and *Nicotiana benthamiana*. Enzyme linked immuno-sorbent assay (ELISA) using general *Potyvirus* antiserum and amplification by reverse transcription polymerase chain reaction (RT-PCR) with degenerate primers indicated that it was a species of *Potyvirus*. It was confirmed as an unknown member of the genus *Potyvirus* by comparing its coat protein sequence with those of other potyviruses. The name Hardenbergia mosaic virus (HarMV) is proposed for this new virus species. Isolates of HarMV were collected from 13 sites, covering much of the natural range of its host. An experimental host range was determined using nine virus isolates tested against plants from 11 species in three families. Its infectivity on three leguminous species important in agriculture (*Lupinus angustifolius*, *L. luteus* and *Trifolium subterraneum*) was established.

The nucleotide (nt) sequences of the coat proteins (CP) of 28 isolates determined there was 24.1- 27.6% diversity with the closest known relative, *Passion fruit woodiness virus* (PWV). Studies of the nucleotide sequences of the CP showed that there was considerable intra-species divergence (mean 13.5%, maximum 20.5%) despite its relatively small geographical distribution and single known natural host. The observed broad diversity strongly suggests long genetic isolation and that HarMV evolved in the region where it was collected. An examination of its phylogeny showed that 28 isolates clustered into eight clades with high bootstrap support (6.2-20.5% inter-clade diversity). Isolates collected at locations distant to the Perth metropolitan area (Margaret River and Seabird) diverged more from isolates collected in the metropolitan area (15.4-21.1% nucleotide sequence diversity). This virus represents the first endemic species to be characterised from Western Australia.

Differences in pathogenicity and symptoms induced on key host species were seen between isolates belonging to different phylogenetic clades. Phylogenetic analysis confirmed the inclusion of HarMV within the Bean common mosaic virus group of the potyviruses and also defined a previously unreported subgroup of six previously described *Potyvirus* species (*Clitoria virus Y*, *Hibbertia virus Y*, PWV, Siratro 1 virus Y, and Siratro 2 virus Y), from Australia, which is further evidence for a prolonged period of genetic isolation.

Both in relation to detection of strains of HarMV, and considering the broader issues of biosecurity and parallel detection of plant viruses, a microarray based detection system was established. To optimise conditions for the development of microarrays for virus detection

poly-L-lysine (PLL) coated microscope slides produced in the laboratory were compared to commercially produced PowerMatrix slides (Full Moon BioSystems). Variables tested for PLL slide production were: choice of printing buffer, probe concentration, method of immobilisation and slide blocking; and in particular the print buffer and immobilisation method had the greatest effect on the quality of PLL microarray slides. Slides printed on PLL surfaces in a high salt buffer (3x Saline sodium citrate) supplemented with 1.5M betaine and immobilised at 42^{o}C overnight retained the highest amounts of probe DNA of the methods tested. Qualitative comparisons of the two showed more probe was retained on PowerMatrix slides which were also more reliable and consistent than the PLL slides.

Probes were designed for eight different virus species and six distinct strains of HarMV to test the potential to use microarrays to distinguish between them. Probes were designed to detect potyviruses at the genus, species and strain levels. Although there was evidence of non-specific hybridisation, the *Potyvirus* array was used to identify six strains of HarMV by hybridisation to species specific probes. Additionally the array was used to identify three other species of *Potyvirus*: *Bean yellow mosaic virus*, PWV and Passiflora foetida virus Y, following amplification with polyvalent PCR primers.

In further microarray tests, using labelled first strand cDNA of *Potato virus X* (PVX) and *Potato virus Y* (PVY) on an array, PVX was strongly detected in leaves known to be infected, but PVY was only weakly detected in infected leaves. Three methods of pre-amplification of virus nucleic acid before hybridisation to the array were investigated to improve the sensitivity of the assay. Two of the methods, Klenow amplification and randomly primed PCR, amplified the target virus; as confirmed by real time PCR. Of the methods tested only randomly primed PCR improved the sensitivity of the microarray. The best amplification method used genus-specific primers with adaptor sequences. This method when tested by real time PCR showed a 3.7Ct reduction for PVX and 16.8Ct for PVY. The microarray correctly identified both viruses.

In this work the first virus (HarMV) endemic to Western Australia was identified, and microarray methods were developed both to identify HarMV and other plant viruses of economic importance. The microarray approach, with further development, may be applicable as a means of identifying incursions of new viruses in a biosecurity situation.

Table of Contents

Presentations and Publications

Some of the results presented in this thesis have been published and presented at scientific meetings.

Publications

Chapter 1:

Webster C.G., Wylie S.J. & Jones M.G.K (2004). "Diagnosis of plant virus pathogens." *Current Science* **86**: 1604-1607.

Chapter 4:

Webster, C. G., Coutts, B. A., Jones, R. A. C., Jones, M. G. K. & Wylie, S. J. (2007). Virus impact at the interface of an ancient ecosystem and a recent agroecosystem: studies on three legume infecting potyviruses in the southwest Australian floristic region. *Plant Pathology* **56**: 729-742.

Wylie, S. J., Webster, C. G., Coutts, B. A., Jones, M. G. K. & Jones, R. A. C. (2007). Hardenbergia mosaic virus, the first indigenous plant virus identified from the Southwest Australian Floristic Region. In ISHS International working group on legume and vegetable viruses: Annual newsletter 2006. Wageningen.

Oral Presentations

Chapter 4:

Australasian Plant Pathology Early Researchers Seminar Series, Perth, 19 October 2007. "An indigenous plant virus from the South West Australasian Floristic Region."

Chapter 5:

6th Australasian Plant Virology Workshop (APVW), Gold Coast, Australia, 31 August – 2 September, 2004 "Towards highly parallel tests for plant virus diagnosis."

Chapter 6:

7th Australian Plant Virology Workshop (APVW), Perth, Western Australia, 8-11 November, 2006. "Identification of unknown viral pathogens using microarrays."

Poster Presentations

Webster C. G., Coutts B. A., Jones R. A. C., Jones M. G. K., Wylie S. J. (2007) "The first indigenous plant virus identified from the South West Australian Floristic Region," Proceedings of the CRC for National Plant Biosecurity Science Exchange, Melbourne, p26.

Webster C.G., Coutts B.A., Jones R.A.C., Jones M.G.K. & Wylie S.J. (2006). "The first indigenous plant virus identified from the South West Australian Floristic Region," Proceedings of the 7th Australasian Plant Virology Workshop, Perth, p12.

Webster C.G., Wylie S.J., Chen G., Kenworthy W. & Jones M.G.K. (2005). "Towards highly parallel plant tests for plant virus diagnosis," Proceedings of the AusBiotech 2005 National Biotechnology Conference, Perth, p20-State Winner of Student Excellence Award.

Webster C.G., Wylie S.J., Chen G., Kenworthy W. & Jones M.G.K. (2004). "Towards highly parallel plant tests for plant virus diagnosis," Proceedings of the Combined ASBMB, ANZSCDB and ASPS Annual Meeting, Perth, p110.

List of abbreviations

Viruses and viroids

Virus and viroid species names are in italics if approved by the International Committee on Taxonomy of Viruses (ICTV) as listed in the 8th ICTV report (Fauquet *et al.*, 2005) or ICTV database (*ICTVdB - The Universal Virus Database*, version 4, April 2006, http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/). Unapproved or tentative names are given in Roman text.

APLV	*Andean potato latent virus*
APMoV	*Andean potato mottle virus*
ApVY	*Apium virus Y*
ApMV	*Apple mosaic virus*
ArMV	*Arabis mosaic virus*
BanMMV	*Banana mild mosaic virus*
BSV	*Banana streak virus*
BaMMV	*Barley mild mosaic virus*
BaYMV	*Barley yellow mosaic virus*
BCMNV	*Bean common mosaic necrosis virus*
BCMV	*Bean common mosaic virus*
BYMV	*Bean yellow mosaic virus*
BtMV	*Beet mosaic virus*
BNYVV	*Beet necrotic yellow vein virus*
BBWV-2	*Broad bean wilt virus 2*
BStMV	*Broome streak mosaic virus*
CCFV	*Cardamine chlorotic fleck virus*
CaLV	Cardamine latent virus
CarVY	*Carrot virus Y*
CYBV	*Cassia yellow blotch virus*
CeMV	*Celery mosaic virus*
CerMV	*Ceratobium mosaic virus*
ChiVMV	*Chilli veinal mottle virus*
CSMV	*Chloris striate mosaic virus*
CSVd	*Chrysanthemum stunt viroid*
CSFV	*Classical swine fever virus*
ClVY	*Clitoria virus Y*
CYVV	*Clitoria yellow vein virus*
ClYVV	*Clover yellow vein virus*
CSV	*Cocksfoot streak virus*
CABMV	*Cowpea aphid-borne mosaic virus*
CFMoMV	*Cucumber fruit mottle mosaic virus*
CGMMV	*Cucumber green mottle mosaic virus*
CMV	*Cucumber mosaic virus*
CABYV	*Cucurbit aphid-borne yellows virus*
CYMMV	*Cymbidium mosaic virus*
DsMV	*Dasheen mosaic virus*
DCMV	Dianella chlorotic mottle virus
DiSMV	Digitara striate mosaic virus
DiVY	*Diuris virus Y*
EAPV	East Asian passiflora virus
EBV	Epstein-Barr virus
EVY	Eustrephus virus Y
FHV	Florida hibiscus virus
FMDV	*Foot-and-mouth disease virus*

GFLV	*Grapevine fanleaf virus*
GFkV	*Grapevine fleck virus*
HarMV	Hardenbergia mosaic virus
HBV	*Hepatitis B virus*
HCV	*Hepatitis C virus*
HiVY	*Hibbertia virus Y*
HAdV-A	*Human adenovirus-A*
CV-A16	Human coxsachievirus A-16
EV-71	Human enterovirus 71
HHV	*Human herpes virus*
HIV	*Human immunodeficiency virus*
HPV	*Human papillomavirus*
HPIV-3	*Human parainfluenza virus 3*
HRSV	*Human respiratory syncytial virus*
HRV-A	*Human rhinovirus A*
HRV-B	*Human rhinovirus B*
HTLV	Human T-cell lymphotrophic virus
INSV	*Impatiens necrotic spot virus*
FLUVA	*Influenza A virus*
JYMV	*Japanese yam mosaic virus*
JGMV	*Johnsongrass mosaic virus*
KSHV	Karposi's sarcoma-associated herpesvirus
KVY	Kennedya virus Y
KYMV	*Kennedya yellow mosaic virus*
KGMMV	*Kyuri green mottle mosaic virus*
LYSV	*Leek yellow stripe virus*
LMV	*Lettuce mosaic virus*
LMoV	*Lily mottle virus*
LASV	Lucerne Australian symptomless virus
MDMV	*Maize dwarf mosaic virus*
MSSV	Maize sterile stunt virus
NVMV	Nicotiana velutina mosaic virus
OMV	*Oat mosaic virus*
ONMV	*Oat necrotic mottle virus*
ORSV	*Odontoglossum ringspot virus*
OYDV	*Onion yellow dwarf virus*
PLDMV	*Papaya leaf distortion mosaic virus*
PRSV	*Papaya ringspot virus*
PSMV	Paspalum striate mosaic virus
PClV	Passiflora chlorosis virus
PCV	Passiflora crinkle virus
PFVY	Passiflora foetida virus Y
PfMV	Passiflora mosaic virus
PFRSV	Passiflora ringspot virus
PaVY	Passiflora virus Y
PCV	Passionfruit crinkle virus
PaMV	Passionfruit mosaic virus
PFMoV	Passionfruit mottle virus
PFMTV	Passion fruit mottle Thailand virus
PWV	*Passion fruit woodiness virus*
PEMV-1	*Pea enation mosaic virus-1*
PSbMV	*Pea seed-borne mosaic virus*
PMMoV	*Pepper mild mottle virus*
PeMoV	*Peanut mottle virus*
PStV	Peanut stripe virus
PSV	*Peanut stunt virus*
PepMV	*Pepino mosaic virus*
PepMoV	*Pepper mottle virus*
PTV	*Peru tomato mosaic virus*
PlVY	*Pleione virus Y*
PPV	*Plum pox virus*
PBRSV	*Potato black ringspot virus*
PLRV	*Potato leafroll virus*

PMTV	*Potato mop-top virus*
PSTVd	*Potato spindle tuber viroid*
PVA	*Potato virus A*
PVM	*Potato virus M*
PVS	*Potato virus S*
PVT	*Potato virus T*
PVV	*Potato virus V*
PVX	*Potato virus X*
PVY	*Potato virus Y*
PYVV	Potato yellow vein virus
PDV	*Prune dwarf virus*
PNRSV	*Prunus necrotic ringspot virus*
PtVY	Pterostylis virus Y
RhoVY	*Rhopalanthe virus Y*
RUBV	*Rubella virus*
RGMV	*Ryegrass mosaic virus*
SarVY	*Sarcochilus virus Y*
ScaMV	*Scallion mosaic virus*
SARS-CoV	*Severe acute respiratory syndrome coronavirus*
S1VY	Siratro 1 virus Y
S2VY	Siratro 2 virus Y
SNMoV	*Solanum nodiflorum mottle virus*
SrMV	*Sorghum mosaic virus*
SAPV	South African passiflora virus
SMV	*Soybean mosaic virus*
SCMoV	*Subterranean clover mottle virus*
SCMV	*Sugarcane mosaic virus*
SCSMV	*Sugarcane streak mosaic virus*
SPFMV	*Sweet potato feathery mottle virus*
SPMMV	*Sweet potato mild mottle virus*
TEV	*Tobacco etch virus*
TMV	*Tobacco mosaic virus*
TMSV	Tobacco mosaic satellite virus
TSV	*Tobacco streak virus*
TVMV	*Tobacco vein mottling virus*
TYDV	*Tobacco yellow dwarf virus*
TAV	*Tomato aspermy virus*
TPMVd	*Tomato planta macho viroid*
TSWV	*Tomato spotted wilt virus*
TYLCV	*Tomato yellow leaf curl virus*
TuMV	*Turnip mosaic virus*
VZV	Varicella-zoster virus
VTMoV	*Velvet tobacco mottle virus*
WMV	*Watermelon mosaic virus*
WSMV	*Wheat streak mosaic virus*
WYMV	*Wheat yellow mosaic virus*
WSSV	*White spot syndrome virus-1*
WPMV	*Wild potato mosaic virus*
WVMV	*Wisteria vein mosaic virus*
YMV	*Yam mosaic virus*
ZGMMV	*Zucchini green mottle mosaic virus*
ZYMV	*Zucchini yellow mosaic virus*

Other abbreviations used in the text

^{o}C	degrees Celsius
3'	hydroxyl-terminus of DNA molecule
5'	phosphate-terminus of DNA molecule
A. A.	amino acid
a. a.	amino allyl
ACT	Australian Capital Territory
ANGIS	Australian National Genome Information Service
BHQ1	black hole quencher 1
BLAST	basic local alignment search tool
bp	base pairs
BSA	bovine serum albumin
CI	cytoplasmic inclusion
cDNA	complementary deoxyribonucleic acid
CF-PCR	competitive fluorescence PCR
COX	cytochrome oxidase
CP	coat (capsid) protein
CSL	Central Science Laboratories, York, UK
C_t	threshold cycle
CTAB	cetyl-trimethylammonium Bromide
C-terminus	carboxy terminus
cv	cultivar
Da	Dalton
DAFWA	Department of Agriculture and Food, Western Australia
DEPC	diethylpyrocarbonate
DIG	digoxigenin
DMSO	dimethyl sulphoxide
DNA	deoxyribose nucleic acid
dNTP	deoxyribose nuceltodie tri-phosphate (mix of A,C,G +T)
ds	double stranded
DTT	dithiothreitol
et al.	et alia, with others.
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
EtOH	ethanol
FAM	5' carboxyfluorescein
FMB	Full Moon Biosystems
fs cDNA	first strand complementary DNA
h	hour
HCl	hydrochloric acid
HC-Pro	helper component-protease
IC-PCR	immunocapture PCR
ICTV	International Committee on the Taxonomy of Viruses
ITS	internal transcribed spaces
IVT	in-vitro transcription
JOE	6-carboxy 4',5'-dichloro-2',7'-dimethoxy fluorescein label
k	kilo
kb	kilobase
KCl	potassium chloride
L	litre
LAMP	loop-mediated isothermal amplification
LB medium	Luria-Bertani medium
LiCl	lithium chloride
LFD	lateral flow device
LNA	locked nucleic acid
µ	micro
µg	microgram
µL	microlitre
µm	micrometre
M	molar
MAb	monoclonal antibody

MALDI-TOF	matrix assisted laser desorption ionization time of flight
MEGA	molecular evolutionary genetics analysis
min	minute
mL	mililitre
mM	millimolar
mRNA	messenger ribose nucleic acid
MS	mass spectrometry
n	nano
NaAc	sodium acetate
$NaBH_4$	sodium borohydride
NaCl	sodium chloride
NaOH	sodium hydroxide
$NaPO_4$	sodium phosphate
ng	nanogram
$(NH_4)_2SO_4$	ammonium sulphate
NIa	*nuclear inclusion a*
NIb	*nuclear inclusion b*
NSW	New South Wales
nt	nucleotide
oligo	oligonucleotide
O/N	overnight
pmol	pico mole
PAb	polyclonal antibody
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pers. comm.	personal communication
Pfu	*Pyrococcus furiosus* DNA polymerase
PLL	poly-L-lysine
QCM	quartz crystal microbalance
Qld	Queensland
RDP	Recombination detection program
RdRp	RNA dependent RNA polymerase
RFLP	restriction fragment length polymorphism
RNA	ribose nucleic acid
ROX	6-carboxy-X-rhodamine
rpm	revolutions per minute
rPCR	random primed polymerase chain reaction
RT-PCR	reverse transcriptase polymerase chain reaction
RTase, RT	reverse transcriptase
s	second
ss	single stranded
SSC	sodium chloride/sodium citrate
SDS	sodium dodecyl sulphate
SWAFR	southwest Australian floristic region
TAE buffer	tris- acetic acid-EDTA electrophoresis buffer
Taq	*Thermus aquaticus* DNA polymerase
TBIA	tissue blot immunoassay
TBE buffer	tris-boric acid-EDTA electrophoresis buffer
TEM	transmission electron microscopy
Tris	tris(hydroxymethyl)aminomethane
UV	ultra violet
vRNA	viral ribose nucleic acid
WA	Western Australia
WGA	whole genome amplification

Code for degenerate oligonucleotides

A	Adenosine	M	AC	V	ACG
C	Cytosine	R	AG	H	ACT
G	Guanine	W	AT	D	AGT
I	Inosine	S	CG	B	CGT
T	Thymine	Y	CT	N	AGCT
U	Uracil	K	GT		

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