Development of a microarray for *Potyvirus* detection and Identification

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Abstract

Potyvirus is the largest and one of the most economically important of the virus genera infecting plants. The complexities of potyvirus identification resulting from many different species, mixed infections, emerging new viruses, new hosts, and new vectors, etc., often requires the use of multiple detection methods which is time consumable and costly. Therefore an assay that can test for a range of potyviruses simultaneously, with good specificity and sensitivity, is desirable. This study looked at the feasibility of producing an oligonucleotide microarray for detection and identification of potyviruses at both species and strain level.

Thirty plant samples with suspected potyvirus infections were collected from field and research laboratories in New Zealand and partial NIb gene, complete CP gene and 3´UTR were sequenced. Twelve definitive potyviruses, one tentative potyvirus, one non-potyvirus, and one novel potyvirus-like sequence were identified, six of which were first records for New Zealand.

Sequence analysis showed that NIb and CP genes and the 3´UTR contained both conserved and variable sequences which were used to design both species and strain level specific probes. Four *Potyvirus* species were chosen for a "proof of concept" study and probes were designed using two different software programs (ROSO and CAG software). A total of eighty five probes including 33 perfect-match and 52 mismatch probes were selected to represent the four targeted potyviruses. Each probe was synthesized with spacers of either 6 or 12 poly-cytosine or poly-thymine at the 5´ terminus. Arrays showed high specificity to the targets when tested using nineteen different geographically diverse potyvirus isolates representing the four target species, four distinct but closely related New Zealand potyviruses, and four healthy plant species. Factors affecting the hybridization efficiency, e.g. the size of the target fragments, secondary structure of probes and targets, label type, strandedness, Tm and GC content of probes, were also investigated.

The approaches and protocols developed in this study should form a useful basis for developing other potyviruses arrays and the results also provide useful insights into issues of generic interest for the development of arrays for detecting other pathogens.

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Abbreviations

A	Adenine
aa	amino acid
agD	Agdia DsMV isolate
Am6C	6 carbon linker arm
ANOVA	one-way-analysis of variance
ATCC	American Type Culture Collection
BCMV	*Bean common mosaic virus*
BLAST	Basic Local Alignment Search Tool
bp	base pairs
BtMV	*Beet mosaic virus*
BYMV	*Bean yellow mosaic virus*
C	cytosine
CAG	Centre of Applied Gensensorik (microarray)
CeMV	*Celery mosaic virus*
ClYVV	*Clover yellow vein virus*
CP	coat protein
CI	cylindrical inclusion
CVMV	*Carnation vein mottle virus*
Cy3	cyanine 3 fluorescent dye
Cy5	cyanine 5 fluorescent dye
D13	~1.3kg PCR product from DsMV
DAG	Asp-Ala-Gly
DC	Dr. Dan Cohen
DNA	deoxyribonucleic acid
Ds	specifc short PCR product from DsMV
dsDNA	double strand DNA
DsMV	*Dasheen mosaic potyvirus*
DSMZ	German Resource Centre for Biological Material
DVY	*Daphne virus* Y
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
eZ	Egypt ZYMV isolate
flD	Florida DsMV isolate
flZ	Florida ZYMV isolate
FreMV	*Freesia mosaic virus*
frP	France PVY isolate
G	Guanine
gP	Germany PVY isolate
GYSV	Garlic yellow streak virus
HC-Pro	helper component protein
hP	Hungary PVY isolate
HyB	hybridization buffer
ICTV	The International Committee on Taxonomy of Viruses
IMMV	*Iris mild mosaic virus*
iP	Italy PVY isolate
ISEM	immunosorbent electron microscopy
iZ	Italy ZYMV isolate
JGMV	*Johnsongrass mosaic virus*
JF	Dr. John D. Fletcher

L1-10	~1.0kb PCR product from New Zealand LYSV isolate 1
L2-10	~1.0kb PCR product from New Zealand LYSV isolate 2
LAMP	loop-mediated isothermal amplification
LBR	Liquid Blocking Reagent
LMV	*Lettuce mosaic virus*
Ls	specifc short PCR product from LYSV
LYSV	*Leek yellow stripe virus*
MacMV	*Maclura mosaic virus*
MAF	Ministry of Agriculture and Forestry
McAb	monoclonal potyvirus antibodies
MM	mismatch
NC	negative control
NeYSV	*Nerine yellow stripe potyvirus*
NIa-Pro	nuclear inclusion a protein
NIa-VPg	nuclear inclusion a linked VPg protein
NIb	nuclear inclusion b protein
nL	The Netherland LYSV isolate
NLV	*Narcissus latent virus*
nP	The Netherland PVY isolate
nt	nucleotide
NVY	Nerine virus Y
NYSV	*Narcissus yellow stripe virus*
OD	optical density
OMV	*Oat mosaic virus*
OrMV	*Ornithogalum mosaic virus*
OrV2	*Ornithogalum virus 2*
OYDV	*Onion yellow dwarf virus*
P7	~0.7kb PCR product from overseas PVY isolates
P8	~0.8kb PCR product from New Zealand PVY
PC	positive control
PCR	polymerase chain reaction
PcAb	polyclonal antisera
pGYSV	purified Garlic yellow streak virus
PK	Pukekohe (a sampling site in Auckland, New Zealand)
poly-A	poly adenines
PM	perfect-match
PMMA	poly methyl methacrylate
PNP	p-nitrophenylphosphate
Ps	specifc short PCR product from PVY
PPV	*Plum pox virus*
PsbMV	*Pea seed-borne mosaic*
PTA	potassium phosphotungstate
PVA	*Potato virus A*
PVY	*Potato virus Y*
PVYg	*Potato virus Y* from garlic
PWV	*Passionfruit woodiness virus*
RNA	ribonucleic acid
RdRp	RNA-dependent-RNA-polymerase
RT	reverse transcription
SCMV	*Sugarcane mosaic virus*
SD	standard deviation
SMV	*Soybean mosaic virus*
SNP	single-nucleotide polymorphism

SpDNA	Salmon Sperm DNA
SPFMV	*Sweet potato feathery mottle virus*
SpMM	Species level mismatched probes
SpS	species-specific
SSCP	Single-strand conformational polymorphism analysis
ssDNA	single strand DNA
StS	strain-specific
T	thymine
TaMV	*Tamarillo mosaic virus*
TBV	*Tulip breaking virus*
tL	Taiwan LYSV isolate
Tm	melting temperature
TMV	*Tobacco mosaic virus*
TuMV	*Turnip mosaic virus*
TV	Tuberose virus
UTR	untranslated region
VPg	viral genome linked protein
WMV	*Watermelon mosaic virus*
Z7	~0.7kb PCR product from ZYMV
ZaMMV	*Zantedeschia mild mosaic virus*
ZaMV	*Zantedeschia mosaic virus*
Zs	specifc short PCR product from ZYMV
ZYMV	*Zucchini yellow mosaic virus*