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Development of a microarray for *Potyvirus* detection and Identification

Ting Wei

PhD Thesis 2008
Development of a microarray
for Potyvirus detection and Identification

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Biological Sciences
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Ting Wei

2008
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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>aa</td>
<td>amino acid</td>
</tr>
<tr>
<td>agD</td>
<td>Agdia DsMV isolate</td>
</tr>
<tr>
<td>Am6C</td>
<td>6 carbon linker arm</td>
</tr>
<tr>
<td>ANOVA</td>
<td>one-way-analysis of variance</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
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<td>BCMV</td>
<td>Bean common mosaic virus</td>
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<td>BLAST</td>
<td>Basic Local Alignment Search Tool</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
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<td>BtMV</td>
<td>Beet mosaic virus</td>
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<td>BYMV</td>
<td>Bean yellow mosaic virus</td>
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<td>D13</td>
<td>~1.3kg PCR product from DsMV</td>
</tr>
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<td>Asp-Ala-Gly</td>
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<tr>
<td>DC</td>
<td>Dr. Dan Cohen</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>Ds</td>
<td>specific short PCR product from DsMV</td>
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<tr>
<td>dsDNA</td>
<td>double strand DNA</td>
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<tr>
<td>DsMV</td>
<td>Dasheen mosaic potyvirus</td>
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<tr>
<td>DSMZ</td>
<td>German Resource Centre for Biological Material</td>
</tr>
<tr>
<td>Dvy</td>
<td>Daphne virus Y</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EM</td>
<td>electron microscopy</td>
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<tr>
<td>eZ</td>
<td>Egypt ZYMV isolate</td>
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<tr>
<td>flD</td>
<td>Florida DsMV isolate</td>
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<tr>
<td>flZ</td>
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<tr>
<td>FreMV</td>
<td>Freesia mosaic virus</td>
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<tr>
<td>frP</td>
<td>France PVY isolate</td>
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<tr>
<td>G</td>
<td>Guanine</td>
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<tr>
<td>gP</td>
<td>Germany PVY isolate</td>
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<tr>
<td>GYSV</td>
<td>Garlic yellow streak virus</td>
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<tr>
<td>HC-Pro</td>
<td>helper component protein</td>
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<tr>
<td>hP</td>
<td>Hungary PVY isolate</td>
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<tr>
<td>HyB</td>
<td>hybridization buffer</td>
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<tr>
<td>ICTV</td>
<td>The International Committee on Taxonomy of Viruses</td>
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<tr>
<td>IMMV</td>
<td>Iris mild mosaic virus</td>
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<tr>
<td>iP</td>
<td>Italy PVY isolate</td>
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<td>ISEM</td>
<td>immunosorbent electron microscopy</td>
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<tr>
<td>JGMV</td>
<td>Johnsongrass mosaic virus</td>
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<tr>
<td>JF</td>
<td>Dr. John D. Fletcher</td>
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</tbody>
</table>
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 specific 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*
Nla-Pro nuclear inclusion a protein
Nla-VPg nuclear inclusion a linked VPg protein
Nlb nuclear inclusion b protein
nL The Netherland LYSV isolate
NLV *Narcissus latent virus*
nP The Netherland PVY isolate
nt nucleotide
N Vy 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 specific 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
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
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<tr>
<td>SpDNA</td>
<td>Salmon Sperm DNA</td>
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<tr>
<td>SPFMV</td>
<td><em>Sweet potato feathery mottle virus</em></td>
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<td>SpMM</td>
<td>Species level mismatched probes</td>
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<td>SpS</td>
<td>species-specific</td>
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<tr>
<td>SSCP</td>
<td>Single-strand conformational polymorphism analysis</td>
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<tr>
<td>StS</td>
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<td>T</td>
<td>thymine</td>
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<td>TBV</td>
<td><em>Tulip breaking virus</em></td>
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<td>Taiwan LYSV isolate</td>
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<td>Tm</td>
<td>melting temperature</td>
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<td>TMV</td>
<td><em>Tobacco mosaic virus</em></td>
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<td>TuMV</td>
<td><em>Turnip mosaic virus</em></td>
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<td>Tuberose virus</td>
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<td>UTR</td>
<td>untranslated region</td>
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<td>VPg</td>
<td>viral genome linked protein</td>
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<td>WMV</td>
<td><em>Watermelon mosaic virus</em></td>
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<td>ZaMMV</td>
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<td>ZaMV</td>
<td><em>Zantedeschia mosaic virus</em></td>
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<td>ZYMV</td>
<td><em>Zucchini yellow mosaic virus</em></td>
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