Detection, Identification and Characterization of New and Emerging Viral and Bacterial Diseases of Ornamental Plants

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SUMMARY

Project Objectives

1. Characterize viruses of major significance to ornamental and nursery crops, including "new" currently uncharacterized or emerging viruses affecting key ornamental crops. **2.** Determine the genome organization of selected important ornamental viruses and develop full-length infectious clones to determine the genes involved in pathogenicity. **3.** Develop improved tools and evaluate methodologies for the identification, detection, and control of bacterial diseases of major significance to woody and floral ornamental crops.

Background

Many plant virus and bacterial diseases cause significant losses in the production and quality of ornamental crops are very difficult to control, and new diseases occur as different crops are introduced or grown in new areas. Many crops are susceptible to multiple pathogens, each of which may cause serious economic losses, and infected plant material may not be acceptable for sale or export. Methods to reliably and rapidly detect and identify these viral and bacterial pathogens are necessary for the production of pathogen-free or pathogen-indexed plants. An understanding of the genome organization of important ornamental viruses and bacteria, as well as the mechanisms of infection and pathogenicity of viruses and bacteria, is needed in order to develop better methods of virus and bacteria disease control. This research is needed to allow increases in both productivity and quality of ornamental plants in an environmentally friendly manner, thereby reducing the use of and reliance on chemical control of pests and diseases.

Accomplishments

Specific examples of recent accomplishments can be found in the 2-page report accompanying this meeting. A few of those will be highlighted here.

Benefits

The ability to detect and identify viruses and bacteria allows selection of pathogen-free plant material for propagation, resulting in higher productivity and quality. Knowledge of the types of viruses affecting a crop also enables implementation of appropriate control measures to prevent introduction or transmission, and permits segregation of susceptible crops. Diagnostic labs may not be able to devote resources and techniques to detect 'new' pathogens.

Ability to receive samples for diagnosis of 'unknown' viruses

- We have an APHIS Diagnostic Permit which allows us to receive samples of plants infected with 'unknown' viruses for testing
- Able to apply multiple techniques to identify unknowns: Electron microscopy, host range, PCR, dsRNA, serology, purification
- Where necessary, able to develop virus-specific reagents for detection

Detection and Identification of 'New' and 'Emerging' Viruses

In addition to what is reported elsewhere in this poster, several other new, unknown or emerging viruses have been detected and at least partially characterized. These include:

- *Bacopa chlorosis virus* (bacopa), *Columbian datura virus* (*Brugmansia*), *Cowpea mild mottle virus* (soybean), *Freesia mosaic virus* (*Freesia*), *Lolium latent virus* (ryegrass), *Omphalodes virus Y* (*Omphalodes*), *Ornithogalum mosaic virus, Ornithogalum necrotic mosaic virus, Ornithogalum stripe mosaic virus* (*Ornithogalum*), *Pepper mild mottle virus* (ornamental pepper), *Spiranthes mosaic virus 3 (Spiranthes)*, *Tricyrtis virus Y* (*Tricyrtis*), *Vallota mosaic virus* (*Cyrtanthus*)
- Discovery and First reports of: *Freesia mosaic virus* (*Spiranthes*); *Lily virus X* (*Tricyrtis*); *Ligustrum necrotic ring spot virus* (Impatiens); and, a 'New Carlavirus' in *Salvia*

Detection and Characterization of a New Potyvirus Causing Flower Break in New Guinea Impatiens

Examples of potyvirus-infected New Guinea Impatiens cultivars exhibiting flower break. (A) Original 'Unknown' "P1" Pink cultivar [light pink to white breaks/streaks on pink flower]; (B) Inoculated 'Sweet Sue' [darker orange streak on orange flower]; (C) 'Magic Sonic Pink'; and, (D) increasing white flower breaking on older plants (younger to older, left to right).

Symptomatic plants were POTY McAb positive; potyvirus 'generic' RT-PCR amplicons were cloned and sequenced; full genome of *Impatiens flower break virus* has been deposited in GenBank.

Develop additional 'broad-spectrum' reagents and methods for detection

One of the best strategies to control virus diseases is the rapid identification and detection of these viruses in plants in quarantine, breeding programs, certification and production. Molecular techniques such as polymerase chain reaction (PCR)-based tests can be very fast and sensitive, and are now used widely to detect these pathogens. In addition to using broad-spectrum reacting antibody tests, PCR using 'generic' primers for a given taxonomic group are very useful in determining the presence of virus(es) in suspect or symptomatic tissues. We will continue to develop:

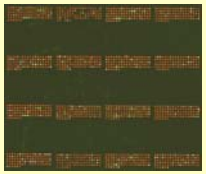
- Group-specific 'Generic' PCR primers
- Cross-reactive monoclonal antibodies (to complement our broad-spectrum Potyvirus McAb; a component of Agdia's POTY Group ELISA Test)
  - Potexviruses and Carlaviruses
- Universal Plant Virus Microarray

Development of a Universal Plant Virus Microarray

- Funded by a USDA-NRI Plant Biosecurity program grant
- Collaboration with: Claude Fauquet (Danforth Center); David Wang (Washington University); Kael Fischer (University of Utah); Ulrich Melcher (Oklahoma State University); and Keith Perry (Cornell University)
- To develop a microarray capable of detection of any plant virus or viroid, including previously uncharacterized viruses, and identification to at least the virus genus level. Based on oligonucleotides representing sequences highly conserved between members of a particular virus genus, or virus species
- Primary usage in quarantine, and in selection of virus-tested nuclear stocks; potential application for initial screening of new introductions (new cultivars, different species) prior to large-scale propagation
- Identification of 'new' viruses using the microarray would enable more rapid development of virus-specific reagents for routine screening of large numbers of plants

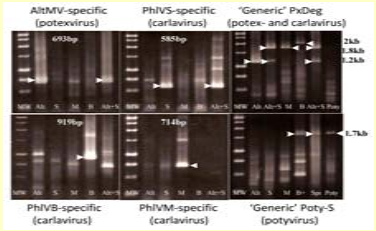
'MiniPlantVirus' Microarray results from a *Pepper mottle virus*-infected test plant

'MiniPlantVirus' microarray has about 750 oligonucleotides representing about 50 viruses, and control oligonucleotides representing healthy plant RNAs



Detection and identification of viruses in *Phlox* species

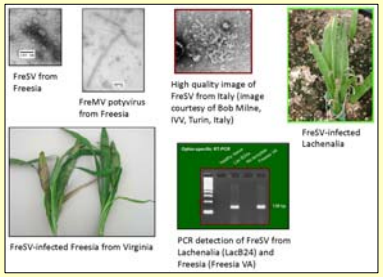
- Utilized broad-spectrum primers to detect: a) potexvirus and/or carlaviruses, and b) potyviruses, in various *Phlox* species
- Developed virus-specific primers for detection and identification of: *Alternanthera mosaic virus* (AltMV; potexvirus); *Phlox virus S* (PhlVS; carlavirus); *Phlox virus B* (PhlVB; carlavirus); *Phlox virus M* (PhlVM; carlavirus); *Angelonia flower break virus* (AnFBV; carmovirus)
- Produced virus-specific antiserum for PhlVS; antiserum for AnFBV (with Scott Adkins, Abed Gera, and Agdia)
- Purified PhlVM for antiserum production
- Assisted nurseries by identification of viruses in production stock



PCR detection of AltMV, PhlVS, PhlVB, PhlVM, and potyviruses affecting phlox. Total RNA extracts from plants infected with various viruses were amplified with virus-specific or broad-spectrum primers. Lanes represent: MW = size markers; Alt = AltMV-infected; S = PhlVS-infected; M = PhlVM-infected; B = PhlVB-infected; Alt+S = mixed infection of AltMV + PhlVS; Poty = potyvirus similar to *Spiranthes mosaic virus 3* (SpiMV3); B+ = mixed infection of PhlVB and Poty; Spi = SpiMV3. Arrowheads indicate diagnostic PCR products.

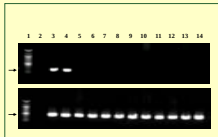
Detection and identification of Freesia sneak virus (FreSV) in Freesia from a Virginia nursery

- Contacted by Mary Ann Hansen, VPI Plant Disease Clinic, to assist with diagnosis of apparent viral disease in Freesia at a commercial nursery
- Performed Electron microscopy (EM) and PCR on Freesia samples, identified presence of FreSV (ophiovirus) – first report in the USA
- Cloned and sequenced coat protein gene, confirming identity of FreSV, and close relationship to isolate from Italy
- Also identified presence of *Freesia mosaic virus* (potyvirus) by EM and sequencing of PCR product
- Co-authored Plant Disease Note with Mary Ann Hansen (Vaira et al., 2009, Plant Disease 93:965)
- Able to detect FreSV from Freesia because we were already working on a FreSV isolate from Lachenalia in material from South Africa (Vaira et al., 2007, Plant Disease 91:770)



Developed a PCR assay that allows simple, fast and sensitive detection and identification of *X. fastidiosa* strains causing oleander leaf scorch disease, and differentiation of oleander strains from other strains of *X. fastidiosa*.

(Huang Q. 2009. Current Microbiology 58: 393-398)



Amplification of *X. fastidiosa* genomic DNA with oleander leaf scorch-specific 'OLS' primer pairs (top gel) and *X. fastidiosa*-specific 'Xf' primer pairs (bottom gel). A 274-bp DNA product (arrow on top) is amplified only in oleander strains (lanes 3 & 4) with the OLS primers, but the 472-bp DNA product (arrow on bottom; using Xf primers) is amplified in all *X. fastidiosa* strains, including oleander, grape, almond, plum, oak, maple, elm, sycamore, and mulberry strains (lanes 3 to 14). Lane 1 - 100-bp DNA ladder; lane 2 - no-template.

Oleander leaf scorch caused by *X. fastidiosa* has become a serious problem throughout the west and southwest including CA, AZ and TX, so specific detection and identification of oleander strains of *X. fastidiosa* is needed to facilitate epidemiological and etiological studies and control of this disease. The specific detection method developed enables studies on the spread and presence of oleander strains in plant hosts and insect vectors, especially in locations where mixed natural infections by oleander and other strains of *X. fastidiosa* occur.

Research on Clove Oil - Significant Antibacterial Activity

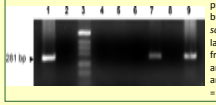
- Discovered that clove oil has significant antibacterial activity *in vitro* against major groups of plant pathogenic bacteria including Gram-negative *Ralstonia, Xanthomonas, Erwinia, Pseudomonas and Agrobacterium*, as well as Gram-positive *Streptomyces* and *Rhodococcus*. [Left figure below]
- Demonstrated that clove oil is effective as a pre-plant soil fumigant to control bacterial wilt of geranium and tomato caused by *R. solanacearum* under greenhouse conditions, thus has the potential to be developed into an environmentally friendly and sound alternative to the use of methyl bromide in integrated disease management of soil-borne plant diseases. [Right below]

Growth of *R. solanacearum* on nutrient agar plates containing 50 ml each of sterile water (right) and clove oil (left) in the center wells 7 days after incubation.

Geranium plants grown in soil pre-treated with: (A) clove oil, (B) clove oil and *R. solanacearum*, or, (C) *R. solanacearum* alone. The picture was taken one month after the soil treatments.

Developed a reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens including *R. solanacearum*.

(Li R, Mock R, Huang Q, Abad J, Hartung JS, and Kinard G. 2008. J. Virological Methods 154: 48-55)



R. Solanacearum was detected in geranium extracts prepared by the modified CTAB method (lanes 7 & 9), but not Qiagen DNeasy kit (lanes 6 & 8). Lanes: 1, *R. solanacearum* genomic DNA; 2, water control; 3, DNA ladder; 4, 6 and 8, DNA extracted with DNeasy kit from a healthy (DI = 0), ≤25% leaves wilted (DI = 1), and a totally wilted plant (DI = 4), respectively; 5, 7 and 9, DNA extracted using the CTAB method from DI = 0, 1 and 4 plants, respectively.

By combining a modified CTAB extraction protocol and a semi-automatic homogenizer, a simple extraction method was developed for reliable detection of pathogens including viruses, viroids, phytoplasma and bacteria. The method allows rapid processing of a large number of samples with low potential of cross contamination, for either diagnostic or research applications.