Oral Session 1

A Scientist's View of Experiences Gained from the USDA FAS-Taiwan COA Joint Research Program: Accomplishments and Impact on Management of Viral Diseases of Ornamental Plants

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ABSTRACT

In 1989, a three-year collaboration project on "Rapid methods for diagnosing ornamental plant viruses and the control measures" among National Chung-Hsing University (NCHU), Taiwan Agricultural Research Institute (TARI) and the Floral and Nursery Plants Research Unit (FNPRU, formerly Floral and Nursery Crops Laboratory) of the U.S. National Arboretum of the United States Department of Agriculture (USDA) was initiated through the USDA Foreign Agriculture Service (FAS)-Taiwan Council of Agriculture (COA) Cooperative Research Program. Subsequently, three additional projects were awarded to Feng-Shan Tropical Horticultural Research Institute, NCHU, TARI, Taichung District Agricultural Improvement Station and Taiwan Seed Propagation Station allowing their investigators to collaborate with scientists at FNPRU. The joint studies are highly productive and have published in various international journals. A novel method for the detection of viruses and phytoplasma in infected plants was developed. Several previously undescribed new viruses infecting ornamental plants were discovered and characterized. A method for efficient production of antigens for immunization was developed and specific rabbit antisera and mouse monoclonal antibodies were produced. Results from USDA FAS-Taiwan COA joint studies have enormous impact on sound management of viral diseases in floral crops as well as the advancement of plant virology.

Key words: Scientist's view, USDA FAS-Taiwan COA Joint Research Program, Management, Viral diseases, Ornamental plant.

INTRODUCTION

For many years through the United States Agency for International Development (USAID), United States-Israel Binational

Agriculture Research and Development Fund (BARD), and Special Foreign Currency Program (PL 480 projects), the Floral and Nursery Plants Research Unit (FNPRU) of the U.S. National Arboretum of the United States Department of Agriculture (USDA) has been working closely with overseas scientists solving plant health problems related to viral diseases. With Taiwan scientists, the collaborations are carried out through the USDA Foreign Agriculture Service (FAS)-Taiwan Council of Agriculture (COA) Cooperative Research Program. The program started in 1968, and in 1989 a three-year collaboration project on "Rapid methods for diagnosing ornamental plant viruses and the control measures" among scientists at National Chung-Hsing University (NCHU), Taiwan Agricultural Research Institute (TARI) and FNPRU (formerly Floral and Nursery Crops Laboratory) was implemented. Subsequently, three additional projects were awarded to Feng-Shan Tropical Horticultural Research Institute, NCHU, TARI, Taichung Agricultural Improvement Station and Taiwan Seed Propagation Station allowing their investigators to collaborate with scientists at FNPRU. The collaborations are highly productive, and our studies have resulted in numerous joint publications in various international journals and reviews. This communication highlights achievements made by investigators on research funded through the USDA-COA Collaborative Research Program.

Accomplishments

1. Calla lily chlorotic spot virus

A new tospovirus, Calla lily chlorotic spot virus (CCSV), was isolated from calla lilies (*Zantedeschia* spp.) (3,9). It causes chlorotic spots, ranging from light green to yellow, on the middle leaves of the affected plants. Virions are about 75 to 105 nm, similar in size to other tospovirus particles reported. CCSV is transmitted by *Thrips palmi*. The virus is serologically but weakly related to *Watermelon silver mottle virus* (WSMoV). Results of reverse transcription-polymerase chain reaction (RT-PCR) show that the conserved regions of the L genes of tospoviruses are present in CCSV. The CCSV S RNA is 3,172 nucleotides (nt) in length, with an inverted repeat at the 5′ and 3′ ends and two open reading frames (ORFs) encoding the nucleocapsid protein (NP) and a nonstructural (NSs) protein in an ambisense arrangement. A typical 3′-terminal sequence (5′-AUUGCUCU-3′) that is shared by all members of the genus *Tospovirus* also is present in the CCSV S RNA. The CCSV NP and NSs protein share low amino acid identities, 20.1 to 65.1% and 19.9 to 66.1%, respectively, with those of reported tospoviruses. Phylogenetic dendrogram analysis indicates that CCSV is a distinct member in the genus *Tospovirus*. The results indicate that CCSV is a new species within the WSMoV serogroup in the genus *Tospovirus*.

2. Tospovirus nucleocapsid proteins

An *in vivo* infectious clone of *Zucchini yellow mosaic virus* (ZYMV) was engineered to express the NPs of tospoviruses *in planta* (5). The open reading frames of NPs of different serogroups of tospoviruses, including *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Watermelon silver mottle virus* (WSMoV), *Peanut bud necrosis virus* (PBNV), and *Watermelon bud necrosis virus* (WBNV), were in frame inserted in frame between the P1 and HC-Pro genes of the ZYMV vector. Six histidine residues and an NIa protease cleavage site were added at the C-terminal region of the inserts to facilitate purification of expressed NPs. Approximately 1.2-2.5 mg NPs per 100 g tissues were purified from leaf extracts of inoculated zucchini squash. Using the expressed WBNV NP, rabbit polyclonal antisera and mouse monoclonal antibodies were produced. The procedure provides a rapid and convenient method for production of large quantities of pure tospoviral NPs *in planta*. The system also has a potential application for the production of other proteins of interest in cucurbits.

3. Calla lily latent virus

A new potyvirus, *Calla lily latent virus* (CLLV) was isolated from apparently healthy calla lilies (*Zantedeschia* spp.) and characterized (2). CLLV infects *Chenopodium quinoa* and develops local lesions on inoculated leaves. Virions are approximately 780 nm long. CLLV is transmitted readily to and established in *C. quinoa*. The virus is, however, transmitted from infected to healthy calla lilies with difficulty. A 1.3-kb cDNA is produced by RT-PCR from CLLV-infected calla lilies and *C. quinoa* using potyvirus degenerate primers. The PCR product was cloned and sequenced. It consists of 1,339 nucleotides (GenBank Accession No. AF469171) corresponding to the genome organization of the 3′ terminal region of potyviruses. The deduced amino acid sequence contains 362 residues comprising the 3′ terminal region of the nuclear inclusion b protein (80 residues) and the complete coat protein (CP) (282 residues). A 253 nt-noncoding region (NCR) is located at the 3′ terminal region of the cDNA. Establishment of CLLV as a new species of *Potyvirus* is based on the uniqueness in the CP gene and 3′ NCR. Polyclonal antibodies produced to *E. coli*-expressed CLLV CP are useful for the detection of CLLV and its CP in calla lilies in enzyme-linked immunosorbent assay (ELISA), sodium dodecyl sulfate (SDS)-immunodiffusion assay, immuno-specific electron microscopy, and western blot. In field surveys, calla lily plants found positive for CLLV by serological methods always remain symptomless throughout the six-month growing season. Occasionally, CLLV is detected in symptomatic calla lilies dually infected with other viruses (*Dasheen mosaic virus* or *Konjak mosaic virus*).

4. New potyviruses associated with golden spider lilies

Two potyviruses associated with golden spider lily severe mosaic disease were identified (1,4). Inoculum prepared from severe mosaic tissue was able to induce local lesions on *Chenopodium quinoa* whereas inoculum prepared from mild mottle tissue failed to infect *C. quinoa*. Tests with 25 known potyvirus antisera failed to detect the presence of viruses in infected spider lilies by ELISA. The antigens, however, reacted with potyvirus-specific monoclonal antibodies. Electron microscopy revealed the presence of flexuous rod-shaped virus particles and pinwheel inclusions in leaf dips and ultra-thin sections of infected severe mosaic tissues, respectively. Purification resulted in typical potyvirus particles measuring about 750 nm. Two species of protein with estimated relative mass of 35 and 37 kDa were detected from samples purified from tissues bearing severe mosaic symptoms. An antiserum was subsequently produced. Following the same purification procedure, only a 37-kDa protein species was detected in the sample purified from mild mottle tissue. In SDS-immunodiffusion

test, antiserum reacted with its homologous antigen prepared from severe mosaic tissues and produced two precipitation bands. It also reacted with the antigen extracted from mild mottle tissue by producing only one single precipitin line. The results indicate that the severe mosaic disease of golden spider lily is caused by two serologically distinct potyviruses and that one, provisionally named *Lycoris mild mottle virus*, induces mild mottle symptoms. The other potyvirus, capable of inducing local lesions on *C. quinoa*, is tentatively named Lycoris potyvirus.

5. Tissue blot immunoassay

A technique involving blotting of tissue on nitrocellulose membranes was described for the detection of plant viruses and a phytoplasma in infected plants (6-8). Tissue blots are prepared by pressing, with a firm and gentle force, the freshly cut tissue surface on nitrocellulose membranes. Antigens in tissue blots on nitrocellulose membranes are detected by enzyme-labeled immunological probes. In indirect immunological methods, the blots are reacted with antigen-specific primary antibodies and detected with enzyme-labeled species-specific secondary antibodies. Alternatively, the blots are reacted with antigen-specific biotintylated primary antibodies and detected with avidin-enzyme conjugates. In direct immunological methods, the blots are reacted and detected with enzyme-labeled antigen-specific antibodies. The unique and novel procedure of tissue blot immunoassay has become one of the standard methods for the investigation of plant pathogens. It enables diagnostic technicians to handle large numbers of test samples. This method has been adopted by an ARS Research Plant Pathologist and applied to virus indexing. It is now utilized in citrus tristeza virus certification in citrus orchards by a private diagnostic laboratory (Nokomis Corp., Altamonte Springs, FL 32715, USA).

6. Engineering Cymbidium mosaic virus resistance in orchids

Full-length heavy and a light chain cDNAs (1545 and 948 bp), CymMV-H 10-1 and CymMV-L 23, respectively, were selected by hybridization with specific oligonucleotide probes (10). The sequence of the CymMV-H 10-1 cDNA clone (GenBank Accession Number AY571287) contains a 5′ untranslated region (37 bp, nt 1-37), an open reading frame (1386 bp, nt 38-nt 1423) including a 19 amino acid leader peptide sequence (57 bp, nt 38-94), a variable region (339 bp, nt 95-433) and a constant region (990 bp, nt 434-1423). The sequence of the 3′ end of this clone contains an untranslated region (102 bp, nt 1424-1525) with a prototype sequence of AATAAA (nt 1492-1497) at a position 28 nucleotides upsteam of the poly A region (nt 1526-1545). The sequence of the CymMV 23 cDNA clone (GenBank Accession Number AY571284) contains part of the 5′ untranslated region (2 bp, nt 1-2), an open reading frame (720 bp, nt 3-722) including a 20 amino acid leader peptide sequence (60 bp, nt 3-62), a variable region 336 bp, nt 63-398) and a constant region (324 bp, nt 399-722). The sequence of the 3′ end of this clone contains an untranslated region (208 bp, nt 723-930) with a prototype sequence of AATAAA (nt 908-913) at a position 17 nucleotides upstream of the poly A region (nt 931-948). The results in this study are a step closer in the exploration of transgenic plants expressing a functional single-chain variable fragment antibody specific to the viral coat proteins for control of CymMV. In addition, a recombinant pBin19 vector containing CymMV coat protein cDNA was transformed into Dendrobium by particle bombardment. Results of transformants are now being evaluated.

CONCLUSION

Early detection and accurate identification of causal agents are among essential strategies for a successful management of diseases caused by viruses. The identity of a specific virus determines the control measures to be adopted, such as the removal and eradication of the source of infection and the control of vector populations in the field and its proximity. Detection of virus infection is usually accomplished by means of serological and/or nucleic acid-based techniques. Results from USDA FAS-Taiwan COA joint studies have enormous impact on sound management of viral diseases in floral crops as well as the advancement of plant virology. Studies led by Shyi-dong Yeh at the Department of Plant Pathology of NCHU are at the forefront in the area of tospovirus research. His laboratory is one of the two leading centers in the world conducting research on tospoviruses. He is a member of the International Working Group of Tospovirus since 1995 when an International Symposium of Tospoviruses and Thrips of Floral and Horticulture Crops was held in Taiwan. Chin-an Chang of Plant Pathology Division at TARI is a vanguard in the research area of ornamental diseases caused by viruses. He has gained recognition among his colleagues and is a member of the Council of International Working Group on Ornamental Viruses since 2000. In 2004, he organized and convened the 11th International Symposium on Virus Diseases of Ornamental Plants in Taiwan. All collaborating investigators actively participated in and presented papers at both symposia. The opportunity of collaboration through USDA FAS-Taiwan COA Cooperative Research Program that increases contact with other scientists throughout the world working in the academic community is a great reward to each scientist involved.

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