台灣農業研究 (J. Taiwan Agric. Res.) 61(1):64–74 (2012)

Identification of Capsicum Chlorosis Virus Causing Chlorotic Spots and Stripes on Calla Lily[1]

Tsung-Chi Chen[2], Chin-An Chang[3], Ya-Chi Kang[4], Shyi-Dong Yeh[4], Chun-Huei Huang[5], and Chin-Chih Chen[5,6]

Abstract

Chen, T. C., C. A. Chang, Y. C. Kang, S. D. Yeh, C. H. Huang, and C. C. Chen. 2012. Identification of Capsicum chlorosis virus causing chlorotic spots and stripes on calla lily. J. Taiwan Agric. Res. 61:64–74.

Calla lily (*Zantedeschia* spp.) is one of the economically important ornamental crops in Taiwan. In a field survey of calla lily conducted during 2005, plants showing symptoms of yellow spots and stripes on leaves were observed in Houli Township, one of the major areas for commercial production of ornamental crops in Taiwan. Fifteen virus isolates were collected from diseased plants of calla lily and purified via three successive local-lesion isolations on leaves of inoculated *Chenopodium quinoa*. A 0.9 kb DNA fragment was amplified from total RNA extracted from all the fifteen virus isolates on infected plants by reverse transcription-polymerase chain reaction (RT-PCR) using the *Tospovirus* genus-degenerate primers gL3637 and gL4510c, designed from the conserved regions of L RNA, revealing that the disease was caused by a *Tospovirus*. The virus isolates reacted positively with the antiserum to the nucleocapsid (N) protein of Capsicum chlorosis virus (CaCV) and the monoclonal antibody to the N protein of *Watermelon silver mottle virus* (WSMoV), indicating that they are members of the WSMoV serogroup. The nucleotide sequences of the N gene of these virus isolates from calla lily were phylogenetically related to CaCV. Furthermore, the pathogenicity of CaCV was also verified by inoculation tests on plants of calla lily.

Key words: Virus disease of calla lily, *Tospovirus*, Capsicum chlorosis virus (CaCV).

Introduction

Calla lily (*Zantedeschia* spp.), belonging to *Araceae*, is a popular ornamental crop in Taiwan and many other countries. Nine viruses have been reported as causal agents of calla lily in Taiwan, including *Calla lily latent virus* (CLLV) (Chen *et al.* 2006b, 2006c), *Dasheen mosaic virus* (DsMV) (Zettler & Hartman 1987, 1995), *Konjak mosaic vi-*

1. Contribution No. 2652 from Taiwan Agricultural Research Institute (TARI), Council of Agriculture. Accepted: February 29, 2012.

2. Associate Professor, Department of Biotechnology, Asia University, Taichung, Taiwan, ROC.
3. Professor and Chair, Graduate Institute of Biochemical Science and Technology, Chaoyang University of Technology, Taichung, Taiwan, ROC.
4. PhD Student and Professor, Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, ROC.
5. Research Assistant and Assistant Researcher, Plant Pathology Division, TARI, Taichung, Taiwan, ROC.
6. Corresponding author, e-mail: chinzue@tari.gov.tw; Fax: (04)23302803.

rus (KoMV) (Pham *et al.* 2002), *Turnip mosaic virus* (TuMV) (Chen *et al.* 2003) and *Zantedeschia mild mosaic virus* (ZaMMV) (Chen *et al.* 2006c; Huang & Chang 2005) of the genus *Potyvirus*; *Cucumber mosaic virus* (CMV) (Zettler & Hartman 1995) of *Cucumovirus*, *Carnation mottle virus* (CarMV) (Chen *et al.* 2002) of *Carmovirus*, *Lisianthus necrosis virus* (LNV) (Chen *et al.* 2006d) of *Tombusvirus*; and Calla lily chlorotic spot virus (CCSV) (Chen *et al.* 2005; Lin *et al.* 2005) of *Tospovirus*.

The genus *Tospovirus*, transmitted by thrips in a persistent manner, is the only plant-infecting genus in the family *Bunyaviridae* (Fauquet *et al.* 2005). The enveloped quasi-spherical particles of tospoviruses are 80 to 110 nm in diameter and contain a tripartite single-stranded (ss) RNA genome denoted large (L), middle (M) and small (S) segments (Fauquet *et al.* 2005). The negative sense L RNA encodes a large RNA-dependent RNA polymerase in the viral complementary (vc) strand for virus replication (de Haan *et al.* 1990). Both M- and S-RNAs are ambisense and each contains two open reading frames (ORFs). The viral (v) strand of M-RNA encodes a nonstructural (NSm) protein for cell-to-cell movement of non-enveloped ribonucleocapsid structures (Kormelink *et al.* 1994; Lewandowski & Adkins 2005), while its vc strand encodes the precursor of Gn and Gc glycoproteins for composing spikes on the viral envelope (Kormelink *et al.* 1992; Law *et al.* 1992). Another nonstructural (NSs) protein encoded by the v strand of S RNA is a gene-silencing suppressor responsible for counteracting the defense mechanisms in plants (Bucher *et al.* 2003; Takeda *et al.* 2002), and forms filamentous inclusion bodies in the infected cells (Kormelink *et al.* 1991). The vc strand of S-RNA encodes the nucleocapsid (N) protein for encapsidation of viral RNAs (de Haan *et al.* 1990).

A threshold of 90% amino acid identity in N protein is the key criterion for demarcation of tospoviruses at species level (Goldbach & Kuo 1996). In addition, tospoviruses can be clustered in serogroups or classified as serotypes on the basis of the serological and phylogenetic relationships of N proteins (Adam *et al.* 1993). The serological-related viruses are grouped as a serogroup, and viruses without serological relationship are designated as monospecies serotypes. So far, there are 22 formal and tentative tospovirus species that have been characterized (Seepiban *et al.* 2011; Zhou *et al.* 2011). Most of them were claded into three major serogroups designating from type members, *Tomato spotted wilt virus* (TSWV), *Watermelon silver mottle virus* (WSMoV) and Iris yellow spot virus (IYSV). Three tospovirus species, *Impatiens necrotic spot virus* (INSV), *Peanut yellow spot virus* (PYSV) and Peanut chlorotic fan-spot virus (PCFV), were classified as monospecies serotypes (Chen *et al.* 2010).

Capsicum chlorosis virus (CaCV), a member of WSMoV serogroup, was first found to infect capsicum and tomato in Queensland, Australia (McMichael *et al.* 2002) and further recognized as a widespread pathogen in solanaceous crops in Thailand (Chiemsombat *et al.* 2008; Knierim *et al.* 2006). Recently, it has become an important quarantine virus for the production of *Phalaenopsis* orchids in Taiwan (Zheng *et al.* 2008). In 2005, symptoms of yellow spots and stripes on leaves of calla lily plants were found in cultivated fields in Houli Township, Taiwan. Fifteen virus isolates were collected from diseased plants by single lesion isolations. All fifteen virus isolates were further identified as isolates of CaCV by sequence determination of their N genes (Chen *et al.* 2007a). In this study, one of calla lily isolates of CaCV (FG1) was back inoculated to calla lily plants to verify that CaCV is the natural causal agent of chlorotic spots and stripes of calla lily. The molecular relationships of the calla lily isolates of CaCV were also analyzed.

Materials and Methods

Virus inoculation

Fifteen isolates of CaCV collected from calla lily in Houli Township, Taichung, Taiwan in 2005 were previously described by Chen *et al.* (2007a). Other viruses used in this study were: one isolate of CaCV from gloxinia, denoted HT-1, which was collected in the United States by Dr. H.-T. Hsu (Hsu *et al.* 2000) and one isolate of WSMoV from watermelon (Yeh *et al.* 1992) and one isolate of CCSV from calla lily (Chen *et al.* 2005) in Taiwan. All tospoviruses were maintained on leaves of *Chenopodium quinoa* and *Nicotiana benthamiana* by mechanical inoculation. A calla lily isolate of CaCV, denoted FG1, was mechanically inoculated on leaves of calla lily seedlings (*Zantedeschia elliottiana* cv. 'Pot of

Gold'). The virus-infected tissues were ground with 0.05 M potassium phosphate buffer (pH 7.5) in a ratio of 1:10 (w/v) and the crude saps were introduced onto leaves of calla lily plants. The inoculated plants were kept in a screenhouse used for further serological and molecular assays.

Indirect enzyme-linked immunosorbent assay (ELISA)

The procedures of indirect ELISA were conducted as previously described (Chen *et al.* 2003; Clark & Adams 1977). The rabbit antisera to the calla lily-infecting viruses were prepared in our laboratory and used for serological tests of viruses, including CarMV, CMV, CLLV, DsMV, KoMV, TuMV and ZaMMV (Chen *et al.* 2006a, 2006c; Chen *et al.* 2007a), the antiserum against the N protein of CaCV HT-1 (Hsu *et al.* 2000), the monoclonal antibodies (MAbs) against the N protein of CCSV and WSMoV (Lin *et al.* 2005) and the *Potyvirus*-general MAb (Agdia, Elkhart, IN). The alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG and the AP-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) were used at a 1:5000 dilution as the secondary antibodies for detecting rabbit and mouse antibodies, respectively. Absorbance at 405 nm (A_{405}) was recorded by PTI max microplate reader (Molecular Devices, Sunnyvale, CA) 30 min after the addition of ρ-nitrophenyl phosphate substrate (1 mg/mL) (Amresco, Solon, OH).

Cloning and sequencing

Extraction of total RNAs of virus-infected plant tissues using the Plant Total RNA Miniprep Purification Kit (Hopegen, Taichung, Taiwan), and reverse transcription (RT) of the first strand cDNA were conducted as described previously (Chen *et al.* 2006b). The primer pairs, gL3637 [5′-CCTTTAACAGT(A/T/G)GAAACAT-3′]/gL4510c [5′-TCATC(A/G)GA(A/G)TG(T/G/C)AC(A/C)ATCCATCT-3′] (Chu *et al.* 2001), designed from the conserved regions of tospoviral L RNAs, and WN2328 (5′-CCATTGGTTTGCCTCCG-3′)/WN3534 (5′-CGTCGACAGAGCAATCGAGGC-3′) (Chen *et al.* 2007a), designed from the S RNA of WSMoV, were used for amplification of a partial L gene fragment and a full-length N gene, respectively. Polymerase chain reaction (PCR) amplification was carried out by 26 cycles: denaturing at 94°C for 1 min, annealing at 50°C for 45 sec, and DNA synthesis at 72°C for 90 sec. An elongation step at 72°C for 6 min was conducted at the last additional cycle. Amplified DNA products were analyzed by electrophoresis in a 1% agarose gel. The amplified DNA fragments corresponding to the full-length N genes were cloned into pCRII-TOPO vector (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. Nucleotide sequences were determined by an automatic DNA sequencer (ABI PRISM 377, Perkin-Elmer, Foster City, CA). Three independent clones were selected for alignment to determine the correction of nucleotide sequences.

Sequence and phylogenetic analyses

Prediction of open reading frames (ORFs) in the determined sequences was conducted using the ORF program of Vector NTI (Invitrogen). Multiple sequence alignments were carried out by the Clustal W program of Vector NTI. The nucleotide sequences were translated into deduced amino acid sequences using the Translate Selection program of Vector NTI. The amino acid sequence alignments were performed by the Align X program of Vector NTI. The phylogenetic relationships among different virus isolates, according to the complete amino acid sequences of N proteins, were also analyzed. The N protein sequences of WSMoV (accession no. U78734), CaCV (accession no. AY036057), CCSV (accession no. AY867502), TSWV (accession no. D13926), INSV (accession no. X66972), and PCFV (accession no. AF080526) were used for comparison. Analyzed sequences were first aligned using Clustal X version 1.8 (Jeanmougin *et al.* 1998). Their phylogenetic relationships were determined using PAUP 4.0 (Swofford, 1998) by the Neighbour-Joining algorithm with the bootstrap resampling method (Felsenstein 1985; Thompson *et al.* 1997). One thousand random resamplings were used to calculate the bootstrap values. The calculated trees were displayed by the TreeView program (Page 1996).

Primer design for virus identification

Specific primers, CaCV4f (5′-TCTACCGTCAGGCAACTTACCGAG-3′) and CaCV777c (5′-ATAATCATCCACAGACAAATTGGCACTAAA-3′), were designed to identify CaCV from the infected plant tissues. RT-PCR was conducted by One-Step RT-PCR Kit (Hopegen, Taichung, Taiwan) as the manufacturer's instruction. The first strand

cDNAs were synthesized at 50°C for 30 min and terminated at 94°C for 2 min, and then PCR was performed by 35 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min. The amplicons were analyzed by electrophoresis in 1% agarose gels.

Results

Isolation and identification of virus from calla lily in Taiwan

In 2005, symptoms of chlorotic spots and stripes accompanying necrosis on leaves of calla lily plants were found in cultivated fields in Houli Township, Taiwan (Fig. 1A). Fifteen symptomatic calla lily samples were collected from field and tested in indirect ELISA. All of the tested samples did not react with the *Potyvirus*-general MAb (Agdia) and the antisera to CarMV, CLLV, CMV, DsMV, KoMV, TuMV and ZaMMV, which are common calla lily-infecting viruses (Chen *et al.* 2003, 2006b; Huang & Chang 2005; Zettler & Hartman 1995). The *Tospovirus* genus-degenerate primers gL3637 and gL4510c (Chu *et al.* 2001) were used in RT-PCR to amplify a 0.9-kb DNA fragment from all samples (Fig. 2). Thus, the calla lily-infecting virus isolates were predicted as tospoviruses. Furthermore, the antiserum to CaCV HT-1 (denoted RAs-CaCV) and the MAbs to CCSV and WSMoV were used in indirect ELISA to verify these calla lily virus isolates. All samples were positively reacted with RAs-CaCV and the MAb to WSMoV (denoted MAb-WSMoV), but not reacted with MAb to CCSV (data not shown). The results demonstrated that the diseased calla lily samples collected from Houli Township were infected by a *Tospovirus* of WSMoV serogroup.

Isolation and back-inoculation of calla lily-infecting tospoviruses

Fifteen virus isolates designated as BM11, BM12, BM13, BM14, BM17, FG1, FG2, FG3, FG13, PG5, PG22, PG23, PG25, PG28 and PG30, were obtained from successive single-lesion isolations on inoculated chenopodium plants. All virus isolates produced similar necrotic local lesions on the inoculated leaves of *C. quinoa* plants (Fig. 1B) and they produced typical symptoms of leaf curl and mosaic on leaves of inoculated plants of *N. benthamiana* (Fig. 1C). The individual virus isolates were confirmed by positively reacting with RAs-CaCV and MAb-WSMoV in indirect ELISA (data not shown). The FG1 isolate was mechanically introduced to ten calla lily seedlings and the results showed that one of the inoculated seedlings showed yellow spots on the newly extended leaf (Fig. 1D). An average ELISA reading of 0.38, two-fold higher than the reading of negative control (0.08), was obtained when the symptomatic calla lily tissue reacted with RAs-CaCV in indirect ELISA to confirm the CaCV infection.

Sequence and phylogenetic analyses of the N genes of the calla lily isolates of CaCV

The primers WN2328 and WN3534, designed from the intergenic region and the 3′-untranslatable region of the S-RNA of WSMoV, respectively, were used to amplify a 1.1-kb DNA fragment from total RNA extract of the aforementioned CaCV isolates. Sequence determination revealed that the amplified DNA fragments correspond to full-length N genes. The N gene sequences of the fifteen virus isolates were submitted to GenBank to obtain accession numbers (Table 1). The N genes of all fifteen CaCV isolates share a high homology of 95.8–97.1% nucleotide sequence identities and 97.5–98.2% amino acid sequence identities with that of the typical CaCV Australian isolate (accession no. AY036057), but a lower homology of 76.7–78.1% nt identities and 84.4–86.2% aa identities with that of the original WSMoV isolate from Taiwan (accession no. U78734). Phylogenetic analysis of the N proteins of fifteen calla lily-infecting CaCV isolates and other tospoviruses revealed that these isolates of CaCV can be divided into two groups: isolates BM17, FG1, FG3, FG13, PG23, PG28 and PG30 were closely related to HT-1 and were clustered as a group, while isolates BM11, BM12, BM13, BM14, FG2, PG5, PG22 and PG25 were clustered as another group (Fig. 3).

Identification of CaCV by RT-PCR

A 0.77-kb DNA fragment was amplified from all tested samples of CaCV isolates by RT-PCR using the primers CaCV4f and CaCV777c, but no signals were obtained from WSMoV (Fig. 4). The results indicated that CaCV can be distinguished from WSMoV by the newly designed specific primers in RT-PCR.

Discussion

In addition to previous reports of *Tospovirus*, such as TSWV (Zettler & Hartman 1995) and CCSV

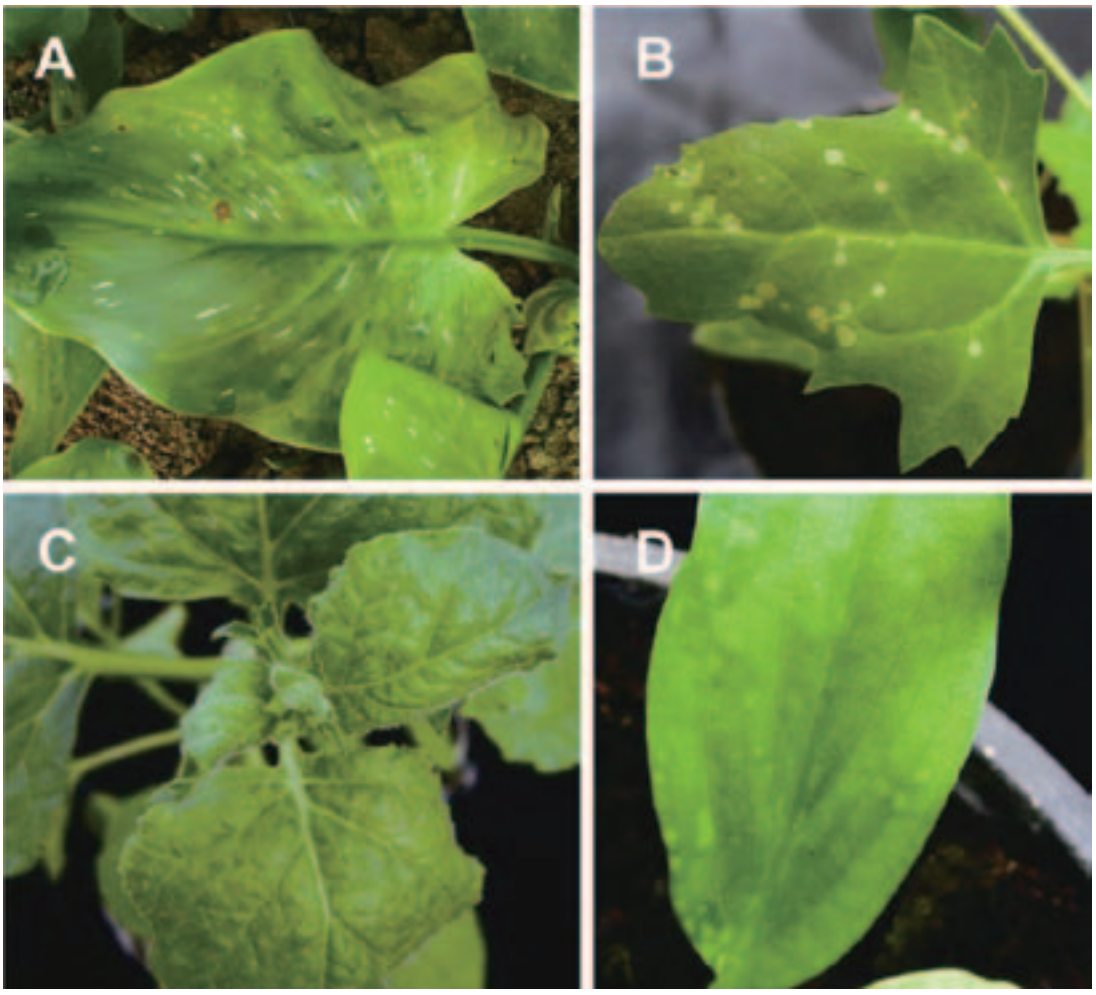

Fig. 1. Symptoms of calla lily-infecting virus isolates on calla lily (A, D), *Chenopodium quinoa* (B) and *Nicotiana benthamiana* (C). (A) Symptoms of yellow spots and stripes on a leaf of a calla lily plant grown in the field; (B) Local lesions produced on a leaf of *Chenopodium quinoa* inoculated with a calla lily-infecting virus isolate FG1; (C) *Tospovirus*-caused chlorotic spot symptoms were observed on the leaves of *Nicotiana benthamiana* inoculated with a single-lesion isolated virus isolate FG1; and (D) Yellow spots produced on a systemic leaf of calla lily inoculated with the FG1 isolate after inoculation for 21 days.

Fig. 2. Detection of fifteen calla lily-infecting virus isolates by reverse transcription-polymerase chain reaction. The degenerate primer pair gL3637/gL4510c (Chu *et al.* 2001), designed from the consensus sequences of tospoviral L RNAs, was used to amplify a 0.9-kb DNA fragment from total RNAs extracted from the naturally infected calla lily samples. Lane M, 100 ladder marker; lane 1 to 15, virus isolates BM11, BM12, BM13, BM14, BM17; FG1, FG2, FG3, FG13; PG5, PG22, PG23, PG25, PG28 and PG30; lane C, Capsicum chlorosis virus (CaCV) gloxinia isolate HT-1; lane W, *Watermelon silver mottle virus* (WSMoV); and lane H, healthy calla lily as negative control.

Table 1. Nucleotide (nt) and amino acid (aa) identities of the nucleocapsid genes of the calla lily-infecting tospovirus isolates compared with those of the typical isolates of Capsicum chlorosis virus (CaCV) and *Watermelon silver mottle virus* (WSMoV)

		Identity (%) to			
		CaCV [z]		WSMoV	
Virus isolate	Accession no.	nt	aa	nt	aa
BM11	EF100597	96.6	97.5	77.7	85.5
BM12	EF095725	95.8	97.5	76.7	84.4
BM13	EF095727	97.0	97.8	78.1	85.8
BM14	EF095726	96.8	98.2	78.1	86.2
BM17	EF100595	96.5	98.2	77.5	85.1
FG1	EF100598	96.4	97.8	77.5	85.5
FG2	EF100600	97.1	97.8	77.9	85.8
FG3	EF100601	96.1	97.5	77.3	85.1
FG13	EF100599	96.2	97.5	77.5	85.5
PG5	EF100596	97.1	97.8	77.9	85.8
PG22	EF100602	96.6	97.8	77.9	85.8
PG23	EF100603	96.4	97.8	77.5	85.1
PG25	EF100604	96.6	97.8	77.9	85.8
PG28	EF100605	96.8	97.8	77.9	85.8
PG30	EF100606	96.0	97.5	77.3	85.1

[z] Accession no. AY036057 for CaCV and accession no. U78734 for WSMoV were used for sequence comparisons.

(Lin *et al.* 2005) on calla lily, this study reveals another *Tospovirus* CaCV responsible for the disease of calla lily occurred in Houli (Taichung, Taiwan) in 2005 was verified to invade calla lily naturally. Diseased calla lily plants infected with CaCV did not mix-infected with other viruses were confirmed by serological tests using various antisera and MAbs to calla lily-infecting viruses and bioassays via single-lesion isolation. Symptoms caused by various viruses on calla lily plants in Taiwan were similar. For instance, symptoms caused by CaCV are similar to those induced by TuMV and CCSV, showing green, yellow or chlorotic spots and strips on the infected leaves of calla lily plants (Chen *et al.* 2003, 2005). For this reason, diagnosis of calla lily virus diseases based on symptoms alone is difficult and unreliable.

Serological and molecular analyses for identification of calla lily-infecting viruses are recommended. Genus broad-spectrum degenerate primers and antibodies are effective tools for preliminary diagnosis of virus diseases in the field. In this study, field samples of diseased calla lily were successfully identified as tospovirus infections by RT-PCR using the *Tospovirus* genus-degenerate primer pair gL3637/gL4510c, which was designed from the consensus sequences of tospoviral L RNAs (Chu *et al.* 2001). It leaded a correct direction to diagnose a virus disease. CaCV was first identified in Australia as a pathogen of *Capsicum* spp. causing chlorotic spots on leaves (McMichael *et al.* 2002), and it was subsequently reported on solanaceous crops in Thailand (Chiemsombat *et al.* 2008) and India (Kunkalikar *et al.* 2010) and on peanut (*Arachis hypogaea* L.) in China (Chen *et al.* 2007b) and Thailand (Chiemsombat *et al.* 2008). In Taiwan, CaCV was reported as a pathogen mainly on tomato (Huang *et al.* 2010) and ornamental crops, such as orchids (Zheng *et al.* 2008), calla lily (Chen *et al.* 2007a), amaryllis (*Hippeastrum hybridum* Hort.) and blood lily (*Haemanthus multiflorus* Martyn.) (Chen *et al.* 2009).

CaCV is genomically closely related to WSMoV with homologies of 84–91% amino acid sequence identities in each viral protein (Knierim *et al.* 2006), and they are serologically indistinguish-

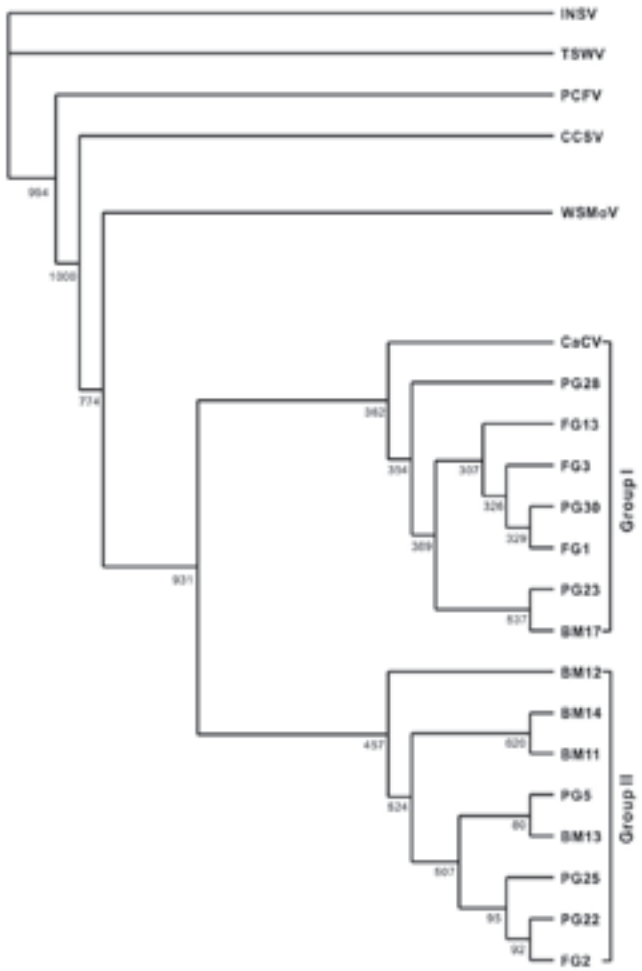

Fig. 3. Phylogenetic analysis of the nucleocapsid (N) proteins of fifteen calla lily-infecting virus isolates and other tospoviruses. The tree was constructed using the neighbour-joining algorithm with the bootstrap resampling method (1000 random resamplings) using PAUP 4.0. Accession numbers of the N protein sequences of the individual virus isolates are listed in Table 1. The N protein sequences of the typical isolates of Capsicum chlorosis virus (CaCV; AY036057), *Watermelon silver mottle virus* (WSMoV; U78734), *Calla lily chlorotic spot virus* (CCSV; AY867502), *Tomato spotted wilt virus* (TSWV; D13926), *Impatiens necrotic spot virus* (INSV; X66972) and *Peanut chlorotic fan-spot virus* (PCFV; AF080526) are used for comparison.

Fig. 4. Design of specific primers for identification of Capsicum chlorosis virus (CaCV) infecting calla lily in reverse transcription-polymerase chain reaction. The primers CaCV4f and CaCV777c were designed to amplify a 0.77-kb DNA fragment from CaCV-infected samples. Lane M, 100 ladder marker; lane 1 to 15, virus isolates BM11, BM12, BM13, BM14, BM17; FG1, FG2, FG3, FG13; PG5, PG22, PG23, PG25, PG28 and PG30; lane C, CaCV gloxinia isolate HT-1; lane W, *Watermelon silver mottle virus* (WSMoV); and lane H, healthy calla lily.

able on the basis of N and NSs proteins (Chen *et al.* 2010). Our results also revealed that CaCV and WSMoV are indistinguishable by RT-PCR analysis using the WSMoV N gene primers WN2328 and WN3534, which were designed for specifically for detection of WSMoV in field survey (Chen *et al.* 2007a). Previous reports also indicate that CaCV, WSMoV, PBNV and WBNV are the four closely related WSMoV-serogroup tospoviruses and are difficult to distinguish by serological assays (Chen *et al.* 2005; Jain *et al.* 2007; Zheng *et al.* 2008). Thus, determination of the full-length N gene sequences is necessary for verifying these tospoviruses.

Development of highly specific primers for RT-PCR amplification is important for detection and identification of viruses. A previously designed primer pair WN2963/WN3469c was reported to identify WSMoV from field samples (Chen *et al.* 2010). Here, a CaCV-specific primer pair CaCV4f/CaCV777c was designed and successfully used in RT-PCR to differentiate CaCV from WSMoV (Fig. 4). These two primer pairs are useful and convenient tools to detect and differentiate CaCV and WSMoV in the fields. In addition to further differentiate the four serologically close-related tospoviruses, such as WSMoV, CaCV, PBNV and WBNV, a convenient one tube-based multiplex RT-PCR method using the combination of individual virus-specific primer pairs will be developed.

No insect vectors were found in the diseased calla lily fields. To our knowledge, WSMoV can be transmitted by *Thrips palmi*, which is a common thrips in Taiwan (Chen *et al.* 1990). However, the reported vector of CaCV, such as *Ceratothripoides claratris* (Premachandra *et al.* 2005), was not found in Taiwan. The category of thrips transmitting CaCV in field should be investigated.

Literature cited

Adam, D., S. D. Yeh, D. V. R. Reddy, and S. K. Green. 1993. Serological comparison of tospovirus isolates from Taiwan and India with impatiens necrotic spot virus and different tomato spotted wilt virus isolates. Arch. Virol. 130:237–250.

Bucher, E., T. Sijen, P. de Haan, R. Goldbach, and M. Prins. 2003. Negative-strand tospoviruses and tenuiviruses carry a gene for a suppressor of gene silencing at analogous genomic positions. J. Virol. 77:1329–1336.

Chen, C. C., C. A. Chang, and S. D. Yeh. 2006a. Molecular characterization and phylogenetic analysis of *Turnip mosaic virus* collected from calla lily. Plant Pathol. Bull. 15:1–8.

Chen, C. C., C. H. Chao, C. C. Chen, S. D. Yeh, H. T. Tsai, and C. A. Chang. 2003. Identification of *Turnip mosaic virus* isolates causing yellow stripe and spot on calla lily. Plant Dis. 87:901–905.

Chen, C. C., T. C. Chen, Y. H. Lin, S. D. Yeh, and H. T. Hsu. 2005. A chlorotic spot disease on calla lilies (*Zantedeschia* spp.) is caused by a tospovirus serologically but distantly related to *Watermelon silver mottle virus*. Plant Dis. 89:440–445.

Chen, C. C., T. C. Chen, S. D. Yeh, H. C. Huang, Y. H. Cheng, and C. A. Chang. 2009. First report of Capsicum chlorosis virus infecting amaryllis and blood lily in Taiwan. Plant Dis. 93:1346.

Chen, C. C., H. T. Hsu, Y. H. Cheng, C. H. Huang, J. Y. Liao, H. T. Tsai, and C. A. Chang. 2006b. Molecular and serological characterization of a distinct potyvirus causing latent infection in calla lilies. Bot. Stud. 47:369–378.

Chen, C. C., H. T. Hsu, F. L. Chiang, and C. A. Chang. 2006c. Serological and molecular properties of five potyviruses infecting calla lily. Acta Hortic. 722:259–270.

Chen, C. C., C. H. Huang, T. C. Chen, S. D. Yeh, Y. H. Cheng, H. T. Hsu, and C. A. Chang. 2007a. First report of Capsicum chlorosis virus causing yellowing stripes on calla lilies. Plant Dis. 91:1201.

Chen, C. C., W. F. Ko, F. J. Jan, C. Y. Lin, and H. T. Hsu. 2002. Characterization of *Carnation mottle* carmovirus isolated from calla lily (*Zantedeschia* spp.). Plant Pathol. Bull. 11:242–243.

Chen, C. C., J. F. Shy, and S. D. Yeh. 1990. Thrips transmission of *Tomato spotted wilt virus* from watermelon. Plant Prot. Bull. 32:331–332.

Chen, K., Z. Xu, L. Yan, and G. Wang. 2007b. Characterization of a new strain of Capsicum chlorosis virus from peanut (*Arachis hypogaea* L.) in China. J. Phytopathol. 155:178–181.

Chen, T. C., Y. Y. Lu, Y. H. Cheng, J. T. Li, Y. C. Yeh, Y. C. Kang, C. P. Chang, L. H. Huang, J. C. Peng, and S. D. Yeh. 2010. Serological relationship between Melon yellow spot virus and *Watermelon*

silver mottle virus and differential detection of the two viruses in cucurbits. Arch. Virol. 155:1085–1095.

Chen, Y. K., F. J. Jan, C. C. Chen, and H. T. Hsu. 2006d. A new natural host of *Lisianthus necrosis virus* in Taiwan. Plant Dis. 90:1112.

Chiemsombat, P., O. Gajanandana, N. Warin, R. Hongprayoon, A. Bhunchoth, and P. Pongsapich. 2008. Biological and molecular characterization of tospoviruses in Thailand. Arch. Virol. 153:571–577.

Chu, F. H., C. H. Chao, M. H. Chung, C. C. Chen, and S. D. Yeh. 2001. Completion of the genome sequence of *Watermelon silver mottle virus* and utilization of degenerate primers for detecting tospoviruses in five serogroups. Phytopathology 91:361–368.

Clark, M. F. and A. N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475–485.

de Haan, P., L. Wagemakers, D. Peters, and R. W. Goldbach. 1990. The S RNA segment of *Tomato spotted wilt virus* has an ambisense character. J. Gen. Virol. 71:1001–1007.

Fauquet, C. M., M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball. 2005. Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press. San Diego. 1162 pp.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Goldbach, R. and G. Kuo. 1996. Introduction: proceedings of the international symposium on tospovirus and thrips of floral and vegetable crops. Acta Hortic. 431:21–26.

Hsu, H. T., P. P. Ueng, F. H. Chu, Y. E. Zhaohui, and S. D. Yeh. 2000. Serological and molecular characterization of a high temperature-recovered virus belonging to *Tospovirus* serogroup IV. J. Gen. Plant Pathol. 66:167–175.

Huang, C. H. and Y. C. Chang. 2005. Identification and molecular characterization of *Zantedeschia mild mosaic virus*, a new calla lily-infecting potyvirus. Arch. Virol. 150:1221–1230.

Huang, C. H., Y. X. Zheng, Y. H. Cheng, W. S. Lee, and F. J. Jan. 2010. First report of Capsicum chlorosis virus infecting tomato in Taiwan. Plant Dis. 94: 1263.

Jain, R. K., S. Bag, K. Umamaheswaran, and B. Mandal. 2007. Natural infection by tospoviruses of cucurbitaceous and fabaceous vegetable crops in India. J. Phytopathol. 155:22–25.

Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins, and T. J. Gibson. 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci. 23:403–405.

Knierim, D., R. Blawid, and E. Maiss. 2006. The complete nucleotide sequence of a Capsicum chlorosis virus isolate from *Lycopersicum esculentum* in Thailand. Arch. Virol. 151:1761–1782.

Kormelink, R., P. de Haan, C. Meurs, D. Peters, and R. Goldbach. 1992. The nucleotide sequence of the M RNA segment of *Tomato spotted wilt virus*, a bunyavirus with two ambisense RNA segments. J. Gen. Virol. 73:2795–2804.

Kormelink, R., E. W. Kitajima, P. de Haan, D. Zuidema, D. Peters, and R. Goldbach. 1991. The nonstructural protein (NSs) encoded by the ambisense S RNA segment of *Tomato spotted wilt virus* is associated with fibrous structures in infected plant cells. Virology 181:459–468.

Kormelink, R., M. Storms, J. van Lent, D. Peters, and R. Goldbach. 1994. Expression and subcellular location of the NSm protein of *Tomato spotted wilt virus* (TSWV), a putative viral movement protein. Virology 200:56–65.

Kunkalikar, S. R., P. Sudarsana, P. Rajagopalan, U. B. Zehr, and K. S. Ravi. 2010. Biological and molecular characterization of Capsicum chlorosis virus infecting chilli and tomato in India. Arch. Virol. 155:1047–1057.

Law, M. D., J. Speck, and J. W. Moyer. 1992. The M RNA of *Impatiens necrotic spot tospovirus* (*Bunyaviridae*) has an ambisense genomic organization. Virology 188:732–741.

Lewandowski, D. J. and S. Adkins. 2005. The tubule-forming NSm protein from *Tomato spotted wilt virus* complements cell-to-cell and long-distance movement of *Tobacco mosaic virus* hybrids. Virology 342:26–37.

Lin, Y. H., T. C. Chen, H. T. Hsu, F. L. Liu, F. H. Chu, C.

C. Chen, Y. Z. Lin, and S. D. Yeh. 2005. Serological comparison and molecular characterization for verification of Calla lily chlorotic spot virus as a new *Tospovirus* species belonging to *Watermelon silver mottle* virus serogroup. Phytopathology 95:1482–1488.

McMichael, L. A., D. M. Persley, and J. E. Thomas. 2002. A new tospovirus serogroup IV species infecting capsicum and tomato in Queensland, Australia. Australas Plant Pathol. 31:231–239.

Page, R. D. 1996. TreeView: an application to display phylogenetic trees on personal computer. Comput. Appl. Biosci. 12:357–358.

Pham, K., S. A. Langeveld, M. E. C. Lemmers, and A. F. L. M. Derks. 2002. Detection and identification of potyviruses in *Zantedeschia*. Acta Hortic. 568: 143–148.

Premachandra, W. T. S. D., C. Borgemeister, E. Maiss, D. Knierim, and H. M. Poehling. 2005. *Ceratothripoides claratris*, a new vector of a Capsicum chlorosis virus isolate infecting tomato in Thailand. Phytopathology 95:659–663.

Seepiban, C., O. Gajanandana, T. Attathom, and S. Attatho. 2011. *Tomato necrotic ringspot virus*, a new tospovirus isolated in Thailand. Arch. Virol. 156:263–274.

Swofford, D. L. 1998. PAUP. Phylogenetic Analysis Using Parsimony. Version 4. Sinauer Associates. Sunderland, MA. 130 pp.

Takeda, A., K. Sugiyama, H. Nagano, M. Mori, M. Kaido, K. Mise, S. Tsuda, and T. Okuno. 2002. Identification of a novel RNA silencing suppressor, NSs protein of *Tomato spotted wilt virus*. FEBS Lett. 532:75–79.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876–4882.

Yeh, S. D., Y. C. Lin, Y. H. Cheng, C. L. Jih, M. J. Chen, and C. C. Chen. 1992. Identification of tomato spotted wilt-like virus infecting watermelon in Taiwan. Plant Dis. 76:835–840.

Zettler, F. W. and R. D. Hartman. 1987. *Dasheen mosaic virus* as a pathogen of cultivated aroids and control of the virus by tissue culture. Plant Dis. 71:958–963.

Zettler, F. W. and R. D. Hartman. 1995. Virus diseases of major ornamental crops-*Dieffenbachia*, *Caladium*, and *Zantedeschia*. p.464–470. *in*: Virus and Virus-like Diseases of Bulb and Flower Crops. (Loebenstein, G., R. H. Lawson, and A. A. Brunt, eds.) Wiley. New York. 556 pp.

Zheng, Y. X., C. C. Chen, C. J. Yang, S. D. Yeh, and F. J. Jan. 2008. Identification and characterization of a tospovirus causing chlorotic ringspots on *Phalaenopsis* orchids. Eur. J. Plant Pathol. 120: 199–209.

Zhou, J., S. K. Kantartzi, R. H. Wen, M. Newman, M. R. Hajimorad, J. C. Rupe, and I. E. Tzanetakis. 2011. Molecular characterization of a new tospovirus infecting soybean. Virus Genes 43:289–295.

Capsicum Chlorosis Virus 引起彩色海芋黃化斑點及條斑之鑑定[1]

陳宗祺[2] 張清安[3] 康雅琪[4] 葉錫東[4] 黃春惠[5] 陳金枝[5,6]

摘 要

陳宗祺、張清安、康雅琪、葉錫東、黃春惠、陳金枝。2012。Capsicum chlorosis virus 引起彩色海芋黃化斑點及條斑之鑑定。台灣農業研究 61:64–74。

彩色海芋 (*Zantedeschia* spp.) 是國內重要之經濟花卉，本研究於2005年在后里地區觀察到彩色海芋植株葉片出現黃色斑點及條斑病徵。取不同罹病植株病葉經由連續三次單斑接種於奎藜 (*Chenopodium quinoa*) 後，共獲得15個純系之病毒分離株。此等病毒分離株之核酸以對應 *Tospovirus* L基因之廣效性引子對gL3637/gL4435c進行反轉錄-聚合酶鏈鎖反應 (Reverse-transcription polymerase chain reaction, RT-PCR)，均可獲得與預估相符約0.9 kb之DNA片段，顯示分離株均為 *Tospovirus*屬病毒之成員。而分離株所產生之單斑均與對應核鞘蛋白 (nucleocapsid protein, NP) 之抗血清包括番椒黃化病毒 (Capsicum chlorosis virus, CaCV) 多元抗體及西瓜銀斑病毒 (*Watermelon silver mottle virus*, WSMoV) 單株抗體產生正反應，顯示其為*Tospovirus*屬WSMoV血清群之成員。經全長核鞘蛋白基因序列及類緣演化分析結果，顯示此等病毒均為CaCV的分離株。進一步完成彩色海芋寄主之回接試驗，病毒分離株可於植株葉片造成與田間觀察到之類似的黃斑病徵，證實CaCV為引起此病害之病毒。

關鍵詞：彩色海芋病毒病、*Tospovirus*屬病毒、番椒黃化病毒。

---

1. 行政院農業委員會農業試驗所研究報告第2652號。接受日期：101年2月29日。
2. 亞洲大學生物科技學系副教授。台灣 台中市。
3. 朝陽科技大學生化科技研究所教授兼所長。台灣 台中市。
4. 國立中興大學植物病理學系博士班學生、教授。台灣 台中市。
5. 本所植物病理組研究助理、助理研究員。台灣 台中市。
6. 通訊作者，電子郵件：chinzue@tari.gov.tw；傳真機：(04)23302803。