Plant Pathology Bulletin 19: 41-52, 2010

Characterization of Melon yellow spot virus Infecting Cucumber (*Cucumis sativus* L.) in Taiwan

Chia-Hung Chao [1, 2], Tsung-Chi Chen [3], Ya-Chi Kang [3], Ju-Ting Li [3], Li-Hsin Huang [4], and Shyi-Dong Yeh [1, 5]

[1] Department of Plant Pathology, National Chung Hsing University, Taichung 402, Taiwan

[2] Crop Environment Section, Taichung District Agricultural Research and Extension Station, Changhua County 515, Taiwan

[3] Department of Biotechnology, Asia University, Wufeng, Taichung County 413, Taiwan

[4] Department of Pesticides Application, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung County 413, Taiwan

[5] Corresponding author, E-mail: sdyeh@nchu.edu.tw; Fax: +886-4-22852501

Accepted for publication: July 12, 2010

ABSTRACT

Chao, C. H., Chen, T. C., Kang, Y. C., Li, J. T., Huang, L. H., and Yeh, S. D. 2010. Characterization of Melon yellow spot virus infecting cucumber (Cucumis sativus L.) in Taiwan. Plant Pathol. Bull. 19: 41-52.

In 2007, a new virus-like disease of cucumber (*Cucumis sativus* L.) was found in a cultivated screenhouse of Tatsuen Township, Changhua County, Taiwan, with symptoms of mosaic on the upper new leaves and yellow spots on the lower leaves, and then neighboring spots fused to form large necrotic spots resulting in necrosis of leaves. A virus isolate TW-C1 was isolated from the diseased cucumber tissues. Cucumber seedlings back-inoculated with this virus displayed symptoms similar to those of the diseased cucumber plants. By electron microscopy, spherical virus particles of 70-110 nm with envelope were observed from the crude extracts of the field samples and back-inoculated cucumber plants. When analyzed with various polyclonal and monoclonal antibodies, the virus TW-C1 only reacted with the monoclonal antibody to the nucleocapsid protein of Melon yellow spot virus (MYSV). Total RNAs extracted from the symptomatic cucumber tissues were used as templates for reverse transcription-polymerase chain reaction (RT-PCR). By RT-PCR, DNA fragments were amplified by the degenerate primer pairs designed from the conserved regions of L and NSm genes of tospoviruses, and MYSV N-gene specific primer pair. Sequences analysis of the amplified DNAs revealed that this virus is an isolate of Melon yellow spot virus (MYSV), denoted as MYSV-TWC1. The N gene of MYSV-TWC1 shares 98-99% nucleotide identities with those of other MYSV isolates reported in GenBank. Transmission of MYSV-TWC1 by *Thrips palmi* was also confirmed. This is the first report of cucumber naturally infected by MYSV in Taiwan.

Keywords: cucumber (*Cucumis sativus* L.), Melon yellow spot virus, *Tospovirus*

INTRODUCTION

Melon yellow spot virus (MYSV) was first noticed from melon (*Cucumis melo* L.) in Shizuoka Prefecture, Japan, and identified as a distinct species of the genus *Tospovirus* [21, 23]. Later, MYSV was also detected from cucumber (*C. sativus* L.) in Kochi Prefecture, Japan [33]. Since then, severe damages caused by MYSV were found on melon and cucumber in Thailand [9], and balsam pear in Japan [34]. In Taiwan, natural infection of cucurbits by several virus species has been reported [1, 16, 19], including *Papaya ringspot virus* (PRSV) [37, 38, 43], *Zucchini yellow mosaic virus* (ZYMV) [1, 17, 25], *Cucumber mosaic virus* (CMV) [1, 16], *Cucumber green mottle mosaic virus* (CGMMV) [1, 16], *Watermelon mosaic virus*-2 (WMV-2) [19], *Melon vein-banding mosaic virus* (MvbMV) [16], *Squash leaf curl Philippines virus* (SLCPHV) [8, 35], *Melon leaf curl virus* (MLCV) [24], *Watermelon silver mottle virus* (WSMoV) [3, 40, 42] and Melon yellow spot virus (MYSV) [5].

The genus *Tospovirus* is the only plant-infecting genus of the family *Bunyaviridae*, naturally transmitted by thrips in a persistent manner. Tospoviruses have quasi-spherical particles of 80-110 nm in diameter with membrane envelope [13] and they infect wide host ranges causing severe damages on many crops globally [30]. The structural nucleocapsid protein (NP) is the most important viral product for identification and diagnosis of a tospovirus. Based on the serological relationship and sequence homology of NPs, the reported 20 formal and tentative tospovirus species have been clustered into three major serogroups with *Watermelon silver mottle virus* (WSMoV), Tomato spotted wilt virus (TSWV) and Iris yellow spot virus (IYSV) as the type members; whereas *Impatiens necrotic spot virus* (INSV), *Peanut yellow spot virus* (PYSV) and Peanut chlorotic fan-spot virus (PCFV) were serologically distinct from any other tospoviruses and thus classified as individual distinct serotypes [6, 20, 15].

In 2007, a field survey of virus diseases on cucurbits was conducted in central Taiwan. A tospovirus-like virus, denoted as TW-C1, was isolated from a cucumber cultivar "Da Feng No. 2" plant (Asusa Spike Seeds, Taipei, Taiwan) in Tatsuen Township, Changhua County, Taiwan. The symptoms on the infected cucumber were similar to those of MYSV-infected melons in Taiwan [5] and melons and cucumbers in Japan [23, 33]. Through the process of single lesion transfer and back inoculation, the causal virus was isolated. Further studies on host range, biological property, serological reactions of NP, reverse transcription-polymerase chain reaction (RT-PCR) and sequence analysis of N gene indicated that the isolated virus is a typical isolate of MYSV. This study represents the first record of MYSV infecting cucumber in Taiwan.

MATERIALS AND METHODS

Virus isolation and back-inoculation

Crude extract was prepared from leaf tissues of a diseased cucumber plant collected from a screenhouse in the field at Tatsuen Township, Changhua County, by grinding in 1:10 (w/v) of 0.01 M phosphate buffer (pH 7.0) containing 0.01 M sodium sulfite. The inoculum was mechanically introduced to the carborundum-dusted leaves of *Chenopodium quinoa* Willd. plants. After three consecutive single-lesion transfers on *C. quinoa*, the virus isolate, denoted as TW-C1, was maintained in *Nicotiana benthamiana* Domin. for further assays. To confirm its role as a causal agent for the disease observed in the field, TW-C1 isolate from an infected *N. benthamiana* plant extracted with the same phosphate buffer was mechanically back-introduced into cucumber (*C. sativus* L.) cultivar "Da Feng No. 2" (Asusa Spike Seeds, Taipei, Taiwan). All inoculated plants were kept in a temperature-controlled greenhouse (25-28°C) for observation of symptom development.

In addition, the watermelon isolate of MYSV, MYSV-TW, collected from Miaoli County of central Taiwan in 2006 [5], a WSMoV isolate (WSMoV-TW) from watermelon [42], a Calla lily chlorotic spot virus (CCSV) from calla lily [2], a Peanut chlorotic fan-spot virus (PCFV) from peanut [10], a high temperature-recovered isolate (HT-1) of Capsicum chlorosis virus (CaCV) isolated from gloxinia in the United States [18], a tomato isolate of *Tomato spotted wilt virus* (TSWV) from New York [39], and an isolate of Iris yellow spot virus (IYSV) from The Netherlands [12] were maintained in plants of *N. benthamiana* and used for comparative studies. Also, ZYMV TW-TN3 originated from Tainan, Taiwan [25] and the PRSV-W Chiayi isolate (PRSV-W-CI) [38] were maintained in plants of *Cucumis metuliferus* (Naud.) and used for parallel assessment.

Electron microscopy (EM)

Slices of leaf tissues (5 × 5 mm^2) from TW-C1-infected cucumber plants were crushed with a toothpick, 10 μl of the sap was mixed with an equal volume of 4% glutaraldehyde (GA) in 0.1 M phosphate solution (pH 7.0) and fixed for 3 min on Parafilm membrane. Formvar-coated, carbon-stabilized copper grids (300 mesh) were floated for 3 min on one drop of crude sap extracted from diseased leaf tissues, rinsed five times with sterile distilled water, stained with 2% uranyl acetate for 10 sec [28], and then observed with a JOEL 200 CX electron microscope (JOEL Ltd., Tokyo, Japan).

Indirect ELISA

To analyze the serological property of TW-C1, indirect ELISA was conducted following the method described previously [11] with some modifications. Four rabbit antisera to ZYMV [25], PRSV [41], CGMMV [1] and CMV [1] and two mouse MAbs to MYSV [6] and WSMoV [26] were used. Clear polystyrene microtitration plates (Greiner Bio-One, Frickenhausen, Germany) were coated with crude extracts diluted to 50-fold in coating buffer (0.05 M sodium carbonate, pH 9.6 and 0.02% sodium azide). The rabbit antisera to each virus were used at a 1:2,000 dilution and the mouse MAbs to each virus were used at a 1:10,000 dilution in conjugate buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.2% ovalbumin) and followed by addition of AP-conjugated goat anti-rabbit and anti-mouse IgGs, respectively, at a 1:5000 dilution in conjugate buffer. The substrate tablets (Sigma-Aldrich, St. Louis, MO) were prepared in the substrate buffer (9.7% diethanolamine and 0.02% sodium azide, pH 9.8) to a final concentration of 1 mg/ml. The level of reaction was recorded as absorbance at 405 nm (A_{405}) using a Bio-Rad Model 680 Microplate reader 30 min after the addition of the substrate solution.

Immunoblotting

Immunoblotting was conducted according to the method described previously [39]. Leaf tissues of uninoculated and TW-C1-infected cucumber and *N. benthamiana* plants were ground in 3 volumes (v/w) of dissociation buffer (100 mM Tris-HCl, pH 7.2, 2% β-mercaptoethanol, 10% sucrose, 0.005% bromophenol blue and 10 mM EDTA). The crude antigens in extracts were electrophoretically separated on a 12% SDS polyacrylamide gel. The proteins were then transferred onto a nitrocellulose (NC) membrane preincubated with TSW buffer (10 mM Tris-HCl, pH 7.4, 0.9% NaCl, 0.25% gelatin, 0.1% Triton X-100 and 2% SDS) for 1 hr. The NC membranes were incubated with the monoclonal antibodies (MAbs) against the NP of MYSV [6] or WSMoV [26] at a 1:10,000 dilution in TSW buffer for 1 hr, followed by the incubation with alkaline phosphatase (AP)-conjugated goat anti-mouse immunoglobulin G (IgG) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1hr (1:5,000 dilution in TSW buffer) and color development by the addition of chromogenic substrates (nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate paratoluidine salt in 100 mM NaCl, 5 mM $MgCl_2$ and 100 mM Tris-HCl, pH 9.5). Extracts from leaves of uninfected plants were used as negative controls.

Reverse transcription-polymerase chain reaction (RT-PCR)

The degenerate primer pairs, t2740 (5'-ATGGG (A/G/T) AT (A/T/G/C) TTTGATTTCATG-3') / t3920c (5'-TCATGCTCAT (C/G) AG (A/G) TAAAT (T/C) TCTCT-3') and tNSm410 (5'-AACTGGAAAAATGATT(T/C) (A/T/C/G) (T/C) TTGTTGG-3') / tNSm870c (5'-ATTAG (C/T) TTGCA (T/G) GCTTCAAT (A/T/G/C)AA(A/G)GC-3'), designed from the conserved regions of the L genes and the NSm genes of tospoviruses, respectively [27], and the species-specific primer pairs, MYSV-N-f (5'-GCCATGGCATG CATGTCTACCGTTACTAAGCTGACA-3') / MYSV-N-r (5'-GTCTAGAGGTACCAACTTCAATGGACTTAG CTCTGGA-3') for the N gene of MYSV [6] and WN2963 (5'-AATAATCGGTGCCAGTCCCCTT-3') / WN3469c (5' -ATGTCTAACGTTAAGCAGCTCACA-3') specific to the N gene of WSMoV [6] were used for RT-PCR. Total RNAs were extracted from TW-C1-infected plant tissues using the Plant Total RNA Miniprep Purification Kit (Hopegen Biotechnology Development Enterprise, Taiwan) and RT-PCR was conducted by One-Step RT-PCR Kit (Hopegen) according to the manufacturer's instructions. The first strand cDNAs were synthesized at 50°C for 30 min and terminated at 94°C for 2 min, and then PCR was carried out by 35 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min.

TA cloning and sequence analyses

The amplified DNA fragments were cloned by the TA cloning kit (Invitogen Corporation, Carlsbad, CA) according to the instruction provided by the manufacturer. DNA fragments were ligated with pCR2.1-TOPO vector (Invitrogen) and transformed into *E. coli* DH5α (Hopegen). Nucleotide (nt) sequences were determined by an automatic DNA sequencing system (AB1377-19; Perkin-Elmer Applied Biosystems, Foster City, CA). The deduced amino acid (aa) sequences were translated using the Sixframe program of Biology WorkBench 3.2 (http://workbench.sdsc.edu). The nt and aa sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/).

Host range test and screening for resistant cucumber cultivars

To characterize the biological properties of the virus isolate TW-C1, plants of 20 species representing six families were mechanically inoculated with the virus. The inoculum was prepared, with the phosphate buffer described above, from leaves of *N. benthamiana* infected with TW-C1 five days post-inoculation (dpi). Inoculated plants were kept in a greenhouse for four weeks to observe the symptom development. The inoculated plants, both symptomatic and asymptomatic, were tested by enzyme-linked immunosorbent assay (ELISA) [11] using the monoclonal antibody (MAb) to the NP of MYSV [6].

Eight commercial cultivars of cucumber provided by different seed companies, including Da Feng No. 2 (Asusa Spike Seeds, Taipei, Taiwan), Ho Sheng May (Ho Sheng Seeds, Tainan, Taiwan), He Tong Cheng Xia (All Lucky Seeds, Harbin Heilongjiang, China), Xi Yan (Known-You Seed, Kaohsung, Taiwan), Xia Hui (Xin He Cheng Seeds, Taipei, Taiwan), Xia Di (Tohoko Seed, Higashi-Machiutsunomiya Tochigi, Japan), Shang Lu (Nong Yi Seeds, Taichung, Taiwan) and Shang Qing (Nong Yi Seeds, Taichung, Taiwan) were tested against MYSV infection. Ten seedlings of each cucumber cultivars were mechanically inoculated with TW-C1. The progress of symptom development was observed daily after inoculation.

Thrips transmission

Virus-free *Thrips palmi* Karny used in this study were obtained from laboratory colonies reared on leaves of bean (*Phaseolus coccineus* L.) at 26°C for over 60 generations. Infected cucumber leaves were cut into small pieces (1.5 × 1.5 cm^2) and placed in a glass vial (diameter 1.5 cm and height 4.5 cm), which was sealed on the top with two layers of Parafilm® (American National Can™, USA). The first instar larvae of virus-free *T. palmi* were transferred in the vial to give a 48 hr acquisition access period (AAP). These AAP thrips larvae were reared on bean leaves at 26°C to the adult stage. Two days after adult emergence, thrips were removed into a setup box and fixed on leaves of healthy cucumber seedlings for an inoculation access period (IAP) of 48 hr. Five to 18 adult thrips were placed on each healthy cucumber seedling. Randomly selected adult thrips individual after IAP was transferred to a 2 ml microcentrifuge tube for RNA extraction using the Total RNA Miniprep Purification Kit (Hopegen Biotechnology Development Enterprise, Taiwan). RT-PCR was conducted using primers MS2315 (5'-GAATGCTCTATTTAAACTTCAATG-3') and MS3186c (5'-CACGTTTCCTAAGTAAACACCATG-3'), designed from the franking sequences of the N gene of MYSV, and then the MYSV N-gene specific primers MYSV-N-f and MYSV-N-r [6] were used for nested PCR. Two weeks after inoculation, the cucumber seedlings were detected by ELISA and RT-PCR, and symptom development was recorded.

RESULTS

Symptoms on cucumber

In the field, diseased cucumber plants showing mosaic symptom on young leaves were noticed. Later, mature leaves developed yellowing, and then neighboring yellow spots fused to form large necrotic spots resulting in gradual necrosis of leaves. The diseased plants with severe symptoms became dwarf with shortened internodes in the late stage (Fig. 1A and B).

Virus isolation and back-inoculation

A virus isolate, denoted TW-C1, was obtained from three times consecutive single-lesion isolation. Necrotic spots on the leaves of *C. quinoa* plants inoculated with TW-C1 were observed 4-5 dpi (Fig. 1C). In addition, TW-C1

was also introduced to *N. benthamiana* plants, on which mosaic and wilting symptoms developed (Fig. 1D). To confirm TW-C1 is the causal agent of the disease observed in field, the virus was back-introduced onto eight cultivars of cucumber seedlings, including Da Feng No. 2, He Sheng No. 1, He Tong Cheng Xia, Xi Yan, Xia Yan, Xia Di, Shang Lu and Shang Qing, by mechanical inoculation. At 10-14 dpi, the inoculated cucumber seedlings of all cultivars showed symptoms of necrotic spots and ringspots on the first inoculated cotyledons, and chlorotic mottle or mosaic symptoms on the newly developed leaves (Fig. 1E). Finally, the upper leaves displayed yellow and necrotic spots

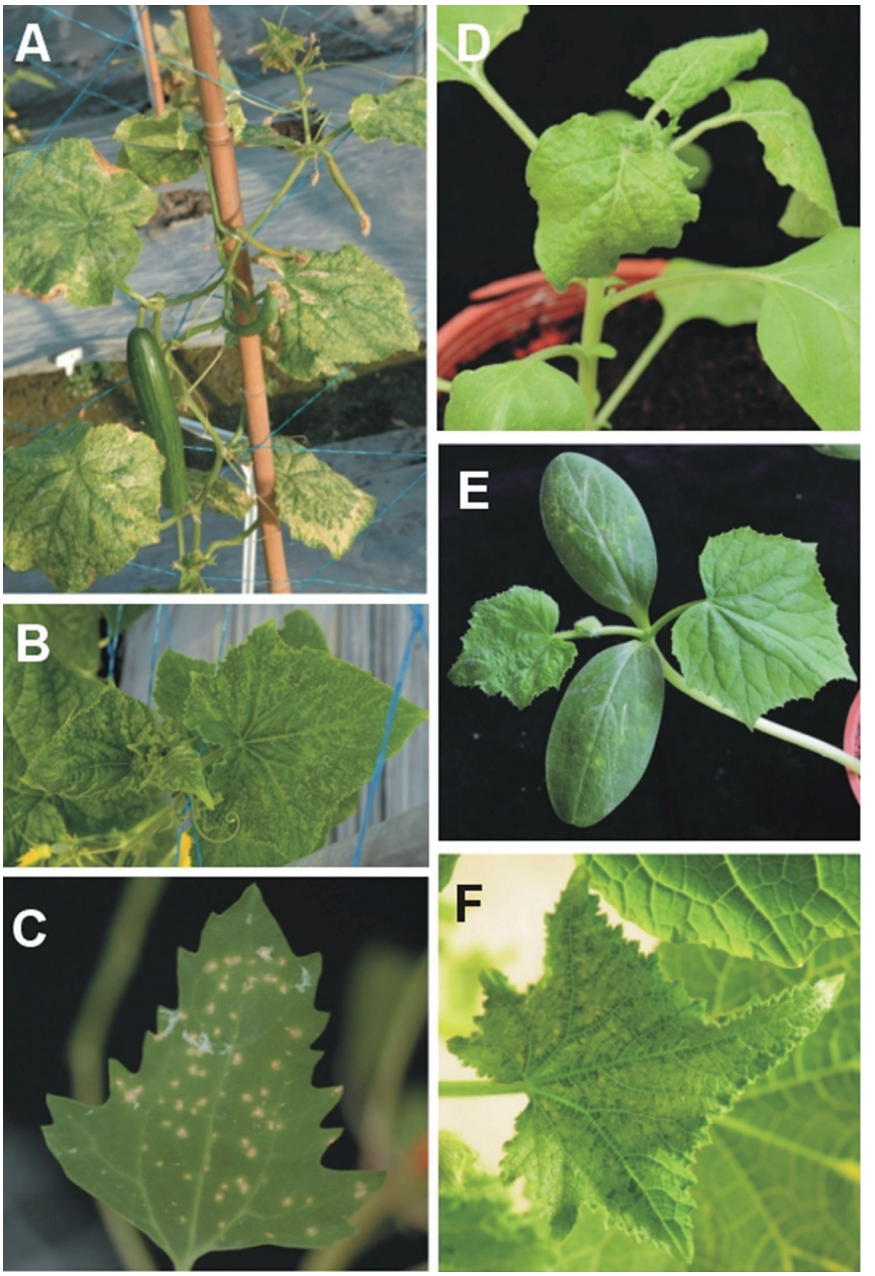

Fig. 1. Symptoms induced by Melon yellow spot virus (MYSV) on cucumber. (A) Necrotic spots, yellowing and mosaic on leaves of a cucumber plant putatively infected with a virus in field screenhouse. (B) Symptoms observed on the upper leaves of an infected cucumber plant. (C) Local lesions induced on a leaf of *Chenopodium quinoa* inoculated with the crude sap extracted from the diseased cucumber plant. (D) Systemic symptoms on a plant of *Nicotiana benthamiana* inoculated with the virus isolate TW-C1 obtained from single-lesion isolation. (E) Cucumber seedlings inoculated with the virus isolate TW-C1 showing yellow spots and mosaic symptoms on cotyledons and true leaves. (F) Mosaic symptoms appeared on a systemic leaf of cucumber seedling after inoculation with the virus isolate TW-C1 by the vector *Thrips palmi*.

symptoms similar to those occurring in nature (Fig. 1A and B). The chlorotic spots gradually covered the whole leaf and finally resulted in necrosis and defoliation.

Virus morphology

The leaf dips prepared from naturally infected cucumber plants were examined by electron microscopy. The presence of roughly spherical virus particles with envelope, measuring 70-110 nm in diameter, was observed. Virus particles of similar size were also observed in the leaf dips prepared from leaves of TW-C1-inoculated cucumber seedlings (Fig. 2). The EM observation predicted that the cucumber virus is a tospovirus.

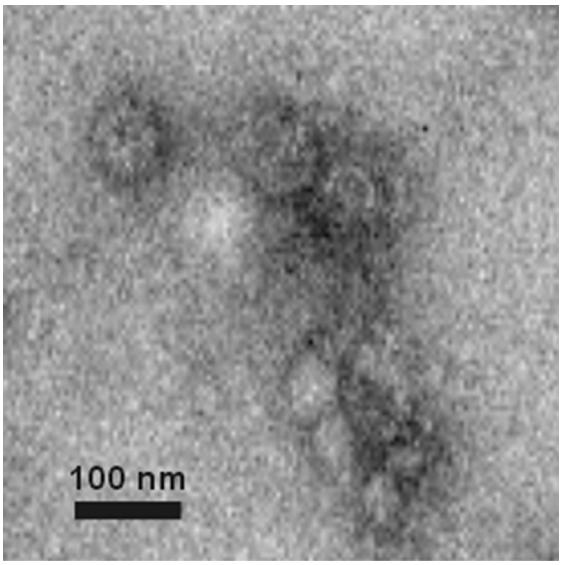

Fig. 2. Transmission electron micrograph of nearly spherical particles with envelope (70-110 nm diameter) in cucumber leaf sap from a TW-C1-inoculated cucumber plant. After fixation with 4% (v/v) glutaraldehyde, leaf sap was placed on a collodion coated grid and negatively stained with 2% (w/v) phosphotungstic acid. The bar represents 100 nm.

Identification of TW-C1 as an isolate of MYSV by serological and sequence analyses

Symptomatic tissues of TW-C1-infected plants were analyzed by serological tests, using several mouse MAbs and rabbit antisera in indirect ELISA for identification of TW-C1. Positive reaction was obtained only when the extract containing the crude antigens of TW-C1 was incubated with the MAb to the NP of MYSV (Fig. 3). No serological responses were obtained when the MAb to the NP of WSMoV and the antisera against the CPs of ZYMV, PRSV-W, CGMMV and CMV were used for reaction with TW-C1.

On the other hand, cucumber seedlings inoculated with TW-C1 were further tested by western blotting. MYSV NP MAb reacted positively with the crude sap of TW-C1-infected cucumber seedlings as well as MYSV-TW-infected samples (Fig. 4). A protein of 30 kDa in the crude sap of TW-C1-infected cucumber plants was regarded as the putative NP of TW-C1.

In addition, the *Tospovirus* genus-universal primer pairs tNSm410/tNSm870c and t2740/t3920c [27] were used for the RT-PCR of total RNAs extracted from the TW-C1-infected samples. The DNA fragments of 0.5 kb and 1.2 kb were amplified by the primer pairs tNSm410/tNSm870c and t2740/t3920c, respectively (Fig. 5A and B). The corresponding amplicons were also obtained from the MYSV-TW- and WSMoV-TW-infected plant tissues. No amplicons were obtained when total RNAs extracted from healthy plants or plants individually infected with other tested tospoviruses. Our results of serological assays

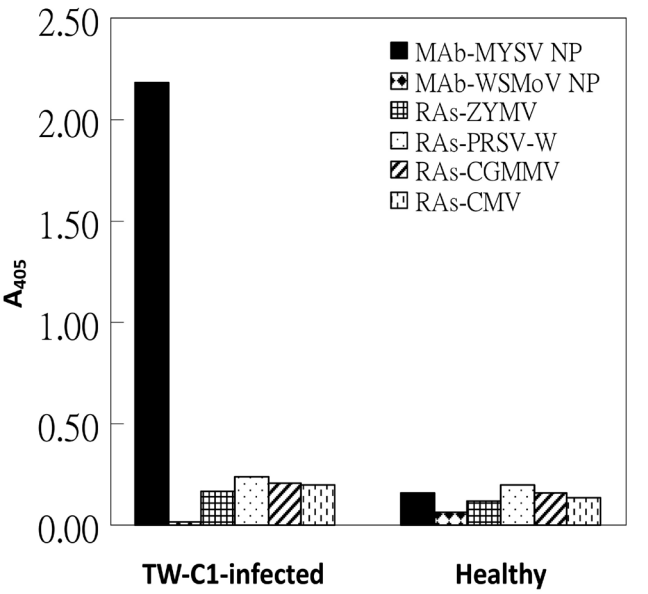

Fig. 3. Enzyme-linked immunosorbent assay of leaf extracts prepared from TW-C1-infected or healthy cucumber plants. Following incubation with the extracts, microfilter plates were individually incubated with specific rabbit antiserum (RAs) to ZYMV [25], PRSV-W [41], CGMMV [1] or CMV [1] and monoclonal antibody (MAb) to MYSV [6] or WSMoV [26], and followed by alkaline phosphatase-labeled goat anti-rabbit and goat anti-mouse immunoglobulin, respectively. Absorbance at 405 nm was recorded 30 min after the addition of the substrate-nitrophenyl phosphate. Readings represent the averages from eight samples.

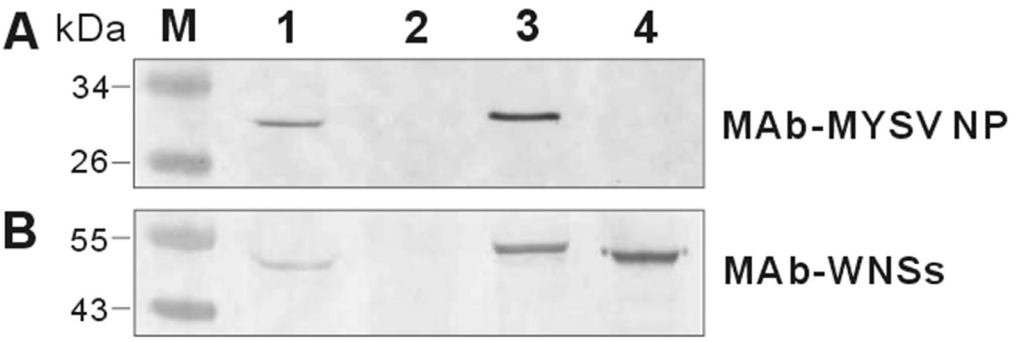

Fig. 4. Western blot analysis of plants infected with TW-C1 using the monoclonal antibody (MAb) to the nucleocapsid protein (NP) of Melon yellow spot virus (MYSV) [6] (A) and the MAb-WNSs to the NSs proteins of *Watermelon silver mottle virus* (WSMoV)-serogroup tospoviruses [4] (B). Equal amounts (5 µl) of extracts from leaf tissues (0.05 g/500 µl) collected at 7 days after inoculation (0.5 cm diameter of disk from three different leaves ground in 500 µl extract buffer) were loaded in each lane. M, prestained protein markers. Lane 1, a cucumber plant infected with TW-C1. Lane 2, an uninfected cucumber plant. Lane 3 and 4, the plants of *Nicotiana benthamiana* infected with MYSV-TW and WSMoV-TW, respectively.

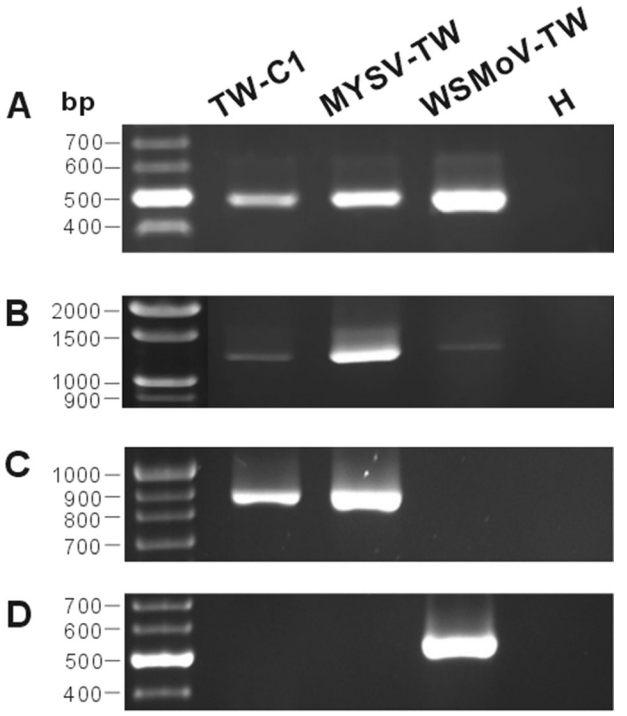

Fig. 5. Detection of the cucumber virus isolate TW-C1 by reverse transcription-polymerase chain reaction (RT-PCR) using *Tospovirus* genus-universal and species-specific primers. (A) Degenerate primer pair tNSm410/tNSm870c designed from the conserved region of NSm genes [27], (B) degenerate primer pair t2740/t3920c designed from the conserved region of L genes [27], (C) specific primer pair MYSV-N-f/MYSV-N-r designed from the nucleocapsid (N) gene of Melon yellow spot virus (MYSV) [6], and (D) specific primer pair WN2963/WN3469c designed from the N gene of *Watermelon silver mottle virus* (WSMoV) [6] were used for RT-PCR. Total RNAs extracted from a healthy plant of *Nicotiana benthamiana* (H) and a plant infected with MYSV-TW or WSMoV-TW were used for comparison.

and RT-PCR indicated that TW-C1 is an isolate of MYSV, denoted as MYSV-TWC1.

In order to more accurately determine the taxonomic status of MYSV-TWC1, the N gene of the virus was amplified by N gene-specific primers MYSV-N-f/MYSV-N-r [6] in RT-PCR (Fig. 5C and D). The amplified DNA fragment of 0.85 kb was cloned and sequenced. Sequence analysis revealed that the N gene of MYSV-TWC1 shares 99% nt identity with that of MYSV-TW, which was collected from watermelon in central Taiwan (accession number FJ386391) [5]. The N gene of MYSV-TWC1 also shares high homology of 98% nt identity with those of the Japan isolates (accession number AB038343 and AB024332) [22, 23] and Thailand isolates (accession number AY673636 and AY574574) [9] of MYSV.

Host range

From the 20 plant species mechanically inoculated, 17 species were susceptible to MYSV-TWC1. Chlorotic spots or necrotic lesions were found on the leaves of the inoculated plants of *Gomphrena globosa* L., *Chenopodium amaranticolor* L., *C. quinoa* Willd., *Benincasa hispida* (Thunb.) Cogn., *Citrullus lanatus* (Thunb.) Matsun & Nakai., *Cucumis metuliferus* E. Mey. ex Naud., *Datura stramonium* L., *Lycopersicon esculentum* Mill., *Nicotiana glutinosa* L., *N. rustica* L., *N. tabacum* cv. Hicks., *Petunia hybrid* Hort. ex Vilm, *Phaseolus vulgaris* L. and *Vigna sesquipedalis* (L.) Fruwirth. Systemic infection of MYSV-TWC1 was observed 6-10 dpi in plants of *B. hispida*, *C. lanatus*, *C. metuliferus*, *C. sativus*, *Cucumis melo* L.,

D. stramonium L., *L. esculentum* and *N. benthamiana* Domin. Symptoms on systemic hosts were mostly mosaic, yellowing, and chlorotic spots turning into necrotic lesions or lines. Infections were also confirmed by positive reactions in ELISA with MAb to the MYSV NP. Plants of *Brassica chinensis* L. and *Raphanus sativus* L. were not infected with MYSV-TWC1, as determined by symptomatology and ELISA (Table 1).

The plants of all the eight cucumber cultivars showed leaf symptoms of necrotic spots, yellow and mosaic two weeks after inoculation with MYSV-TWC1. The inocula prepared from the symptomatic plants yielded more than 100 local lesions per leaf on *C. quinoa*. The results indicated that all cucumber cultivars tested are susceptible to MYSV-TWC1. The infections were also confirmed by positive reactions in ELISA with MAb to the MYSV NP.

Thrips transmission

A total of 15 cucumber seedlings were treated with *T. palmi* vectors previously fed on MYSV-TWC1-infected plants in duplicate experiments. Four treated plants became diseased, showing mosaic and yellowing on the systemic leaves (Fig. 1F). Crude extracts of the leaves of these symptomatic plants positively reacted with MYSV NP MAb in indirect ELISA, verifying the infection with MYSV-TWC1 (Fig. 6A). Moreover, a DNA fragment of 0.85 kb, corresponding to the N gene of MYSV-TWC1, was amplified from total RNAs extracted from 10 of 14 adult thrips individuals randomly collected from the treated plants after 48 hr AAP at the larvae stage and 48 hr IAP at the adult stage (Fig. 6B). Our results indicated that *T. palmi* is able to transmit MYSV-TWC1.

DISCUSSION

Classification of tospoviruses is difficult due to their high variability and wide host range. Host reactions, serological relationships of NP and vector specificity are critical for identification of tospovirus species [14]. During the surveys conducted in central Taiwan, a virus-like

Table 1. Host range of a tospovirus-like virus (TW-C1) isolated from diseased cucumber in central Taiwan

Family	Species	Symptoms*	
		Inoculated leaves	Upper leaves
Amaranthaceae	*Gomphrena globosa* L.	NS	−
Brassicaceae	*Brassica chinensis* L.	−	−
	Raphanus sativus L.	−	−
Chenopodiaceae	*Chenopodium amaranticolor* L.	YS	−
	C. quinoa Willd.	NS, YS	−
Cucurbitaceae	*Benincasa hispida* (Thunb.) Cogn.	Y, M	M
	Citrullus lanatus (Thunb.) Matsun & Nakai.	Y, M	M,NS
	Cucumis metuliferus E. Mey. Ex Naud.	Y, M	M
	C. sativus L.	Y, M	M
	C. melo L.	Y, M	M
Solanaceae	*Datura stramonium* L.	CS	M, NS
	Lycopersicon esculentum Mill.	YS	M, NS
	Nicotiana benthamiana Domin.	YS, M	M
	N. glutinosa L.	NS	−
	N. rustica L.	NS	−
	N. tobacum cv. Hicks.	NS	−
	Petunia hybrid Hort. ex Vilm	YS	−
Leguminosae	*Phaseolus vulgaris* L.	YS	−
	Vigna sesquipedalis (L.) Fruwirth	NS	−

* Symptoms are abbreviated as follows: NS, necrotic spots; CS, chlorotic spots; M, mosaic; Y, yellowing; YS, yellow spot; −, no symptom.

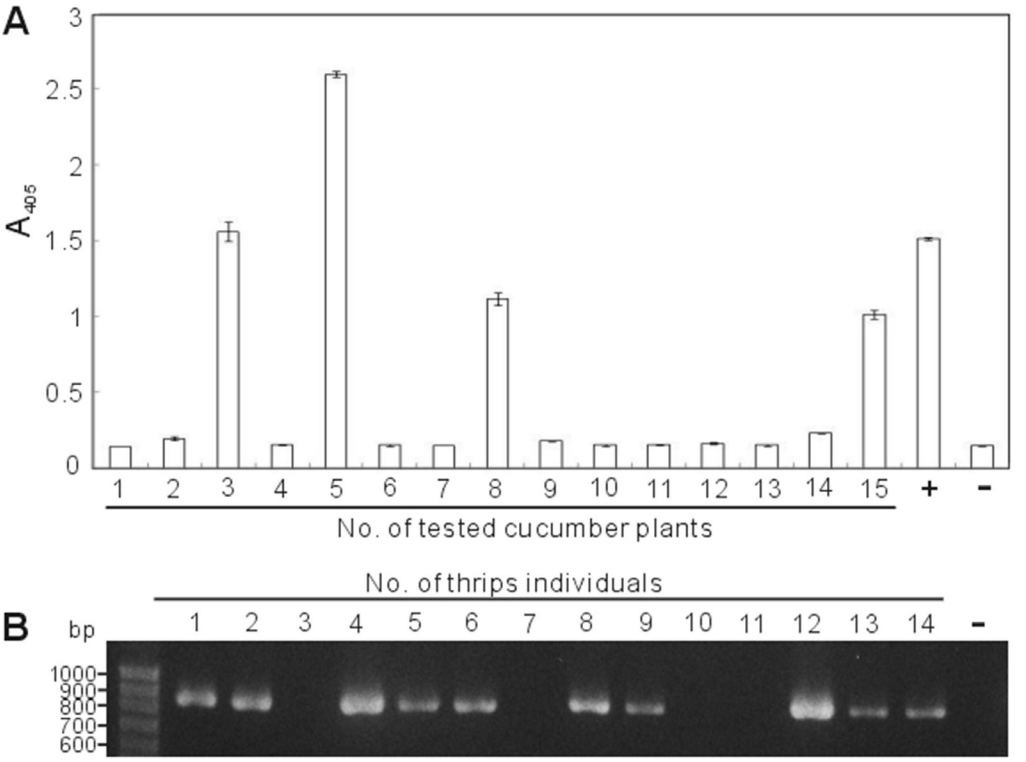

Fig. 6. Confirmation of symptomatic cucumber plants infected with MYSV-TWC1, isolated from cucumber, by *Thrips palmi* transmission. (A) Indirect enzyme-linked immunosorbent assay was conducted using the monoclonal antibody specific to MYSV for detection of MYSV from the plants treated with the vector. The crude extract from a diseased cucumber plant mechanically inoculated with MYSV-TWC1 was used as the positive control (+). The crude extract from an uninfected cucumber plant was used as the negative control (-). (B) The adult thrips individuals after 48 hr IAP treatment during the larvae stage and 48 hr IAP at the adult stage on treated plants were randomly collected for detection for the presence of MYSV-TWC1 by nested RT-PCR. An amplified fragment of 0.85 kb corresponding to the N gene of the virus is shown. Total RNA extracted from a virus-free *T. palmi* reared at laboratory was used as the negative control (-).

disease causing necrotic spots, yellow and mosaic on leaves of cucumber was noticed and the causal agent, denoted as TW-C1, was isolated in 2007. Spherical enveloped particles of TW-C1, 70 to 110 nm in diameter, typical of tospoviruses, were observed by EM. RT-PCR amplification using the *Tospovirus* genus broad-spectrum degenerate primer pairs [27] revealed that TW-C1 is a member of the genus *Tospovirus*. Although MYSV was reported to react with the antiserum prepared to WSMoV NP in western blotting [23] and ELISA [5], these two tospoviruses can be unambiguously distinguished by the MAb to MYSV or WSMoV produced by our laboratory [6]. In serological analyses, TW-C1 crude antigens reacted specifically with the MAb to the MYSV NP. Our results indicated that TW-C1 is an isolate of MYSV, thus denoted as MYSV-TWC1.

To further verify this tospovirus, the MYSV N gene-specific primers MYSV-N-f and MYSV-N-r were used to amplify a DNA fragment of 0.85 kb by RT-PCR from total RNA extracted from MYSV-TWC1-infected plants. WSMoV N gene-specific primers WN2963 and WN3469c used in RT-PCR failed to amplify any DNA fragment from the total RNA of MYSV-TWC1-infected samples. The RT-PCR results confirm the results of serological tests and indicate that the specificity of our produced MAb in ELISA is comparable to RT-PCR. Comparison of the N gene sequence of MYSV-TWC1 with those of the different isolates of MYSV showed that MYSV-TWC1 is closely related to MYSV-TW, another Taiwan isolate collected from watermelon in 2006 [5], with 99% nt identity, and also to the Japanese [22, 23] and Thai [9] isolates, both with 98% nt identity.

The melon thrips, *T. palmi*, was reported as the vector for several members of the genus *Tospovirus*, including WSMoV [7], PBNV [36], CCSV [2], MYSV [21] and TSWV [29]. The vector is prevailing in Taiwan and is an important insect vector for transmitting cucurbit-infecting WSMoV on

the island. Thrips transmission experiment was conducted in this study and our results showed that *T. palmi* is able to transmit MYSV-TWC1. This is the first report to prove that *T. palmi* is the transmission vector of MYSV in Taiwan.

MYSV appears to spread throughout Taiwan now and is considered an emergent limiting factor for the production of melon [(1)]. In Japan, 398 cucumber accessions originating from 26 countries were evaluated by mechanical inoculation with melon and cucumber isolates of MYSV, and no accessions immune or highly resistant to these MYSV isolates were found [(31)]. The previous study showed that high temperature (25°C and 30°C) facilitated symptom expression and viral spread in the cucumber accessions [(32)]. Our screening for resistance to MYSV from the eight commercial cucumber cultivars in Taiwan also indicated that they are all susceptible to MYSV-TWC1. Since all of the commercial cultivars tested are F1 hybrids, their parental lines need to be further tested. In addition, cucumber lines introduced from foreign countries also need to be screened for possible resistant or tolerant character for the control of MYSV.

ACKNOWLEDGEMENTS

This study was supported in part by the projects 96AS-14.1.2-BQ-B1 and 97AS-14.1.2-BQ-B1 from the Council of Agriculture and NSC96-3111-P-225-001-Y from the National Science Council of Taiwan.

LITERATURE CITED

1. Chang, Y. M., Hsaio, C. H., Yang, W. Z., Hseu, S. H., Chao, Y. J., and Huang, C. H. 1987. The occurrence and distribution of five cucurbit viruses on melon and watermelon in Taiwan. J. Agri. Res. 36: 389-397.
2. Chen, C. C., Chen, T. C., Lin, Y. H., Yeh, S. D., and Hsu, H. T. 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.
3. Chen, C. C., Ho, H. M., Chang, T. F., Chao, C. H., and Yeh, S. D. 1995. Characterization of a tospovirus-like virus isolated from wax gourd. Plant Prot. Bull. 37: 117-131.
4. Chen, T. C., Huang, C. W., Kuo, Y. W., Liu, F. L., Hsuan Yuan, C. H., Hsu, H. T., and Yeh, S. D. 2006. Identification of common epitopes on a conserved region of NSs proteins among tospoviruses of *Watermelon silver mottle virus* serogroup. Phytopathology 96: 1296-1304.
5. Chen, T. C., Lu, Y. Y., Cheng, Y. H., Chang, C. A., and Yeh, S. D. 2008. Melon yellow spot virus in watermelon: a first record from Taiwan. Plant Pathol. 57: 765-765.
6. Chen, T. C., Lu, Y. Y., Cheng, Y. H., Li, J. T., Yeh, Y. C., Kang, Y. C., Chang, C. P., Huang, L. H., Peng, J. C., and Yeh, S. D. 2010. Serological relationship between Melon yellow spot virus and *Watermelon silver mottle virus* and differential detection of the two viruses in cucurbits. Arch. Virol. DOI 10.1007/s00705-101-0688-y.
7. Chen, C. C., Shy, J. F., and Yeh, S. D. 1990. Thrips transmission of tomato spotted wilt virus from watermelon. Plant Prot. Bull. 32: 331-332.
8. Cheng, Y. H., Liao, J. Y., Deng, T. C., Tsai, C. H., and Hu, C. C. 2009. Prevalence of Squash leaf curl Philippines virus occurred on melon plants in south Taiwan. Plant Path. Bull. 18: 78.
9. Chiemsombat, P., Gajanandana, O., Warin, N., Hongprayoon, R., Bhunchoth, A., and Pongsapich, P. 2008. Biological and molecular characterization of tospoviruses in Thailand. Arch. Virol. 153: 571-577.
10. Chu, F. H., Chao, C. H., Peng, Y. C., Lin, S. S., Chen, C. C. and Yeh, S. D. 2001. Serological and molecular characterization of peanut chlorotic fan-spot virus, a new species of the genus *Tospovirus*. Phytopathology 91: 856-863.
11. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.
12. Cortes, I., Livieratos, I. C., Derks, A., Peters, D., and Kormelink, R. 1998. Molecular and serological characterization of iris yellow spot virus, a new and distinct tospovirus species. Phytopathology 88: 1276-1282.
13. Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U., and Ball, L. A., eds. 2005. Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier/ Academic Press, San Diego, CA. USA. 1259 pp.
14. Goldbach, R., and Kuo, G. 1996. Introduction: Proceedings of the international symposium on tospovirus and thrips of floral and vegetable crops. Acta Hortic. 431: 21-26.
15. Hassani-Mehraban, A., Botermans, M., Verhoeven, J. T., Meekes, E., Saaijer, J., Peters, D., Goldbach, R., and Kormelink, R. 2010. A distinct tospovirus causing necrotic streak on *Alstroemeria* sp. in Colombia. Arch. Virol. 155: 423-428.

16. Hseu, S. H., Huang, C. H., Chang, C. A., Yang, W. Z., Chang, Y. M. and Hsiao, C. H. 1987. The occurrence of five viruses in six cucurbits in Taiwan. Plant Prot. Bull. 29: 233-244.
17. Hseu, S. H., Wang, H. L., and Huang, C. H. 1985. Identification of a Zucchini yellow mosaic virus from *Cucumis astivus*. J. Agri. Res. China 34: 87-95.
18. Hsu, H. T., Ueng, P. P., Chu, F. H., Ye, Z. H., and Yeh, S. D. (2000). Serological and molecular characterization of a high temperature-recovered virus belonging to tospovirus serogroup IV. J. Gen. Plant Pathol. 66: 167-175.
19. Huang, C. H., Liang, S. C., Deng, T. C., and Hseu, S. H. 1993. Comparison of diagnostic hosts and serological tests for four cucurbit potyvirus. Plant Pathol. Bull. 2: 169-176.
20. Jan, F. J., Chen, T. C., and Yeh, S. D. 2003. Occurrence, importance, taxonomy, and control of thrips-borne tospoviruses. Pages 391-411 in: Advances in Plant Disease Management. H. C. Huang and S. N. Acharya, eds. Research Signpost, Trivandrum, India.
21. Kato, K., Hanada, K., and Kameya-Iwaki, M. 1999. Transmission mode, host range and electron microscopy of a pathogen causing a new disease of melon (*Cucumis melo*) in Japan. Ann. Phytopathol. Soci. Japan 65: 624-627.
22. Kato, K. and Hanada, K. 2000. Characterization of the S RNA segment of melon yellow spot virus. Japan. J. Phytopathol. 66: 252-254.
23. Kato, K., Hanada, K., and Kameya-Iwaki, M. 2000. Melon yellow spot virus: A distinct *species* of the genus *Tospovirus* isolated from melon. Phytopathology 90: 422-426.
24. Liao, J. Y., Deng, T. C., Hu, C. C., and Cheng, Y. H. 2009. Discussion of melon leaf curl disease occurred in south Taiwan. Plant Path. Bull. 18: 77-78.
25. Lin, S. S., Hou, R. F., Huang, C. H., and Yeh, S. D. 1998. Characterization of *Zucchini yellow mosaic virus* (ZYMV) isolates collected from Taiwan by host reactions, serology, and RT-PCR. Plant Prot. Bull. 40: 163-176.
26. Lin, Y. H., Chen, T. C., Hsu, H. T., Liu, F. L., Chu, F. H., Chen, C. C., Lin, Y. Z., and Yeh, S. D. 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.
27. Lin, Y. P. 2007. Comparison of the ambisense M RNA and negative sense L RNA of Calla lily chlorotic spot virus with those of other tospoviruses. Master Thesis. Department of Plant Pathology, National Chung Hsing University.
28. Milne, R. G. 1970. An electron microscope study of tomato spotted wilt virus in sections of infected cells and in negative stain preparations. J. Gen. Virol. 6: 267-276.
29. Moyer, J.W. 2000. Tospoviruses. Pages 592-597 in Encyclopedia of Microbiology. Vol 4. R. Hull ed. Academic Press. London.
30. Pappu, H. R., Jones, R. A. C., and Jain, R. K. 2009. Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. Virus Res. 141: 219-236.
31. Sugiyama, M., Okuda, M., and Sakata, Y. 2009a. Evaluation of resistance to melon yellow spot virus in a cucumber germplasm collection. Plant Breed. 128: 696-700.
32. Sugiyama, M., Yoshioka, Y., and Sakata, Y. 2009b. Effect of temperature on symptom expression and viral spread of melon yellow spot virus in resistant cucumber accessions. J. Gen. Plant Pathol. 75: 381-387.
33. Takeuchi, S., Okuda, M., Hanada, K., Kawada, Y., and Kameya-Iwaki, M. 2001. Spotted wilt disease of cucumber (*Cucumis sativus*) caused by melon yellow spot virus. Japan J. Phytopathol. 67: 46-51.
34. Takeuchi, S., Shimomoto, Y., and Ishikawa, K. 2009. First report of melon yellow spot virus infecting balsam pear (*Momordica charantia* L.) in Japan. J. Gen. Plant Pathol. 75: 154-156.
35. Tsai, W. S., Shih, S. L., Green, S. K., and Jan, F. J. 2007. Occurrence and molecular characterization of squash leaf curl Phillipines virus in Taiwan. Plant Dis. 91: 907.
36. Vijayalakshmi, K. 1994. Transmission and ecology of *Thrips palmi* Karny, the vector of peanut bud necrosis virus. Dissertation, Andhra Pradesh Agricultural University.
37. Wang, H. L., Wang, C. C., Chiu, R. J., and Sun, M. H. 1978. Preliminary study on papaya ringspot virus in Taiwan. Plant Prot. Bull. 20: 133-140.
38. Wang, J. J., and Yeh, S. D. 1998. Characterization of the papaya ringspot virus W type isolates collected from different areas of Taiwan by host reactions, immunodiffusion tests and RT-PCR. Plant Prot. Bull. 40: 383-395.
39. Yeh, S. D., Chao, C. H., Cheng, Y. H., and Chen, C. C. 1996. Serological comparison of four distinct tospoviruses by polyclonal antibodies to purified nucleocapsid proteins. Acta Hortic. 431: 122-134.
40. Yeh, S. D., Cheng, Y. H., Jih, C. L., Chen, C. C., and Chen, M. J. 1988. Identification of tomato spotted infecting horn melon and watermelon. Plant Prot. Bull. 30: 419-420. (Abstract)
41. Yeh, S. D., and Gonsalves, D. 1984. Evaluation of

induced mutants of Papaya ringspot virus for control by cross protection. Phytopathology 74: 1086-1091.
42. Yeh, S. D., Lin, Y. C., Cheng, Y. H., Jih, C. L., Chen, M. J., and Chen, C. C. 1992a. Identification of tomato spotted wilt-like virus on watermelon in Taiwan. Plant Dis.76: 835-840.
43. Yeh, S. D., Jan, F. J., Chiang, C. H., Doong, T. J., Chen, M. C., Chung, P. H., and Bau, H. J. 1992b. Complete nucleotide sequence and genetic organization of papaya ringspot virus RNA. J. Gen. Virol. 73: 2531-2541.

摘　要

趙佳鴻[1, 2]、陳宗祺[3]、康雅琪[3]、李如婷[3]、黃莉欣[4]、葉錫東[1, 5]. 2010. 在台灣危害胡瓜之甜瓜黃斑病毒之特性. 植病會刊 19: 41-52. ([1]台中市國立中興大學植物病理學系；[2]彰化縣大村鄉台中區農業改良場；[3]台中縣霧峰鄉亞洲大學生物科技學系；[4]台中縣霧峰鄉農業藥物毒物試驗所；[5]聯絡作者，電子郵件：sdyeh@nchu.edu.tw；傳眞：+886-4-2285-2501)

2007 年於彰化縣大村鄉所種植之胡瓜發現一種類似由病毒引起葉片產生壞疽斑點、黃化、嵌紋病徵之植株。病徵通常於新生葉片出現嵌紋病徵，隨病勢進展在較老葉片上出現黃化病斑，鄰近斑互相癒合成大型塊斑，黃化後期逐漸轉爲壞疽，導致全葉壞疽。罹病組織經單斑分離及將所分離之病毒株系 TW-C1 回接健康胡瓜苗出現與田間發現之相同病徵。 罹病葉片粗汁液經以電子顯微鏡觀察到直徑 70-110 nm 之球形具有套膜之病毒顆粒。再以各種多元及單元抗體經酵素連接免疫吸附反應 (enzyme-linked immunosorbent assay，ELISA) 及電泳免疫轉漬分析 (immunoblotting)，證實其僅和洋香瓜黃斑病毒 (Melon yellow spot virus，MYSV) 之核鞘蛋白單元抗體反應。 以 *Tospovirus* 屬 M RNA 和 L RNA上高保留性區域序列設計之二組簡併性引子及以病毒核鞘基因序列設計專一性引子進行反轉錄聚合酶連鎖反應 (reverse transcription-polymerase chain reaction，RT-PCR) 進行偵測，結果顯示此 TW-C1 病毒株系爲 *Tospovirus* 屬之洋香瓜黃斑病毒 (Melon yellow spot virus，MYSV)，故將其命名爲 MYSV-TWC1。利用反轉錄聚合酶連鎖反應增幅 MYSV-TWC1 核鞘蛋白基因 (nucleocapsid gene) 並定序，將所得之核鞘蛋白基因序列與基因庫 (GenBank) 中已發表之Tospovirus屬各病毒核鞘蛋白基因序列進行比對分析，經比對後與 MYSV 已發表各分離株間具 98-99% 之核苷酸序列相同度 (nucleotide identity)。以南黃薊馬 (*Thrips palmi*) 進行傳播試驗，亦證實此薊馬可傳播 MYSV-TWC1。綜合以上試驗結果鑑定引起台灣胡瓜壞疽斑點、黃化及嵌紋的病毒爲 *Tospovirus* 屬之 MYSV，本研究爲台灣首次發現 MYSV 可感染胡瓜之報告。

關鍵詞：胡瓜、甜瓜黃化斑點病毒、番茄斑萎病毒屬