Identification of Tomato Spotted Wilt-like Virus on Watermelon in Taiwan

SHYI-DONG YEH, YING-CHUN LIN, YING-HUEI CHENG, CHUNG-LUNG JIH, and MOH-JIH CHEN, Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; and CHING-CHUNG CHEN, Taichung District Agricultural Improvement Station, Tatseun, Changhua County, Taiwan, Republic of China

ABSTRACT

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The causal agent of an unusual disease affecting watermelon (*Citrullus lanatus*) in Taiwan was identified as tomato spotted wilt-like virus in the tospovirus group on the basis of particle morphology, host reactions, serology, and transmission by thrips. Host symptoms included leaf crinkling, mottling, yellow spotting, short internodes, upright growth of younger branches, and tip necrosis. The systemic infection of cucurbits and transmissibility by the vector *Thrips palmi* distinguished this isolate from most other tomato spotted wilt virus (TSWV) isolates. Serological tests by enzyme-linked immunosorbent assay and western blotting with polyclonal and monoclonal antibodies demonstrated that the watermelon virus is serologically related to the TSWV that causes silver mottle on watermelon in Okinawa (Japan) and not to the tomato, lettuce, and amaryllis isolates of TSWV from other countries.

During the growing season of 1988, an unusual disease occurred in watermelon fields in Changhua county in central Taiwan. This region is considered the most important watermelon production area of the country. Foliar symptoms of affected plants included mottling, crinkling, yellow spotting, and narrowing of leaf laminae. Plants were severely stunted and exhibited short internodes, upright growth of younger branches, tip necrosis, and dying back (Fig. 1A). Fruit set was reduced, and fruit that developed remained small, malformed, and showed necrotic spots or silver mottling (Fig. 1B).

Several viruses cause diseases in watermelon in Taiwan. These include cucumber green mottle mosaic virus, cucumber mosaic virus, W strain of papaya ringspot virus, and zucchini yellow mosaic virus (9). However, none of the symptoms usually caused by these viruses was similar to those observed on the affected plants in Changhua county. Furthermore, antisera to the above-mentioned viruses did not react with sap from infected watermelon specimens. The purposes of this investigation were to identify and characterize the causal agent of a previously unreported viral disease of *Citrullus lanatus* (Thunb.) Matsum. & Nakai in Taiwan. In this paper, we present evidence, using host range studies, electron microscopy, and thrips transmission, that the virus affecting watermelon in Taiwan was a tomato spotted wilt-like virus in the tospovirus group. This watermelon isolate is serologically related to the watermelon silver mottle isolate found in Japan (12), but differs from L-serotype or I-serotype TSWV isolates reported from the other geographical areas of the world (6,16).

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MATERIALS AND METHODS

Virus isolation and host reactions. Extracts from the leaf tissue of diseased watermelon plants collected from Tacheng in Changhua county were mechanically inoculated on plants of *Chenopodium quinoa* Willd., *Petunia hybrida* Vilm., *Nicotiana benthamiana* Domin., and *Datura stramonium* L.; an inoculation buffer (0.01 M phosphate buffer containing 0.01 M sodium sulfite, pH 7.0) was used. After three single-lesion transfers on *C. quinoa*, the virus was maintained in *N. benthamiana* and *D. stramonium* for further assays. We also transferred the virus from *N. benthamiana* back to healthy watermelon, *C. lanatus* 'Fubao No. 2', to confirm its role as a causal agent for the disease observed in the field. This watermelon virus isolate was tentatively designated TSWV-W for further study. A typical isolate of the common type (=L-serotype [6]) of TSWV isolated from tomato in New York State, designated TSWV-NY (provided by R. Provvidenti, Cornell University, Geneva, New York), was used for comparison throughout the study.

To characterize the biological properties, we used several differential hosts (Table 1) of TSWV for the assays. Tissue from *N. benthamiana* infected with TSWV-W was ground in cold inoculation buffer, and extracts were rubbed on test plants previously dusted with 600-mesh Carborundum. At least 10 plants of each species or cultivar were inoculated with TSWV-W or TSWV-NY. In cases that yielded negative or uncertain results, we repeated the tests at least twice, and we assayed the test plants on the local lesion host, *C. quinoa*, to check for the presence of the virus. The plants were kept in a screenhouse at 24–38 C and observed for 4 wk or longer.

Electron microscopy. Slices of leaf tissue (3 × 5 mm) of *N. benthamiana* infected with TSWV-W were triturated in 4% glutaraldehyde and fixed for 10 min. The sap was then mixed with an equal volume of 2% phosphotungstic acid (PTA) in 0.01 M phosphate buffer (pH 7.0) for 1 min and transferred to Formar-coated grids for examination with a JEOL 200 CX electron microscope.

Because it was difficult to observe the virus in the crude sap of back-inoculated watermelon stained by PTA in the method described above, the crude sap was stained by uranyl acetate. Slices of leaf tissue (5 × 5 mm) from infected watermelon plants were crushed with a toothpick, and 10 μl of the sap was placed on Parafilm membrane. Formar-coated grids were then placed on top of drops for absorbing for 30 sec, rinsed three times with sterile distilled water, stained with two drops of 2% uranyl acetate, each for 10 sec, and observed with the electron microscope.

For ultrathin sectioning, leaf slices (1 × 3 mm) from lesions of *C. quinoa* infected with TSWV-W were prefixed with 2.5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.2) and postfixed with 1% osmium tetroxide, each for 2 hr at 4 C. After dehydration with ethanol, the samples were imbedded in LR White (Polysciences, Inc., Warrington, PA) (14). Ultrathin sectioning was performed with glass knives mounted on a model Ultracut E ultratome (Reichert-Jung, C. Reichert Optische Werke AG, Austria). Sections were stained with 2% uranyl acetate for 15 min, rinsed with distilled water, and restained with 0.5% lead citrate for 1–2 min. The samples were finally examined in a JEOL 200 CX electron microscope.

Thrips transmission. The watermelon disease was closely associated with the insect *Thrips palmi* Karny, which is the most important thrips for damaging watermelon in Taiwan. Initial experiments to determine if field-collected *T. palmi* could transmit TSWV-W to the test plants were conducted. Adult thrips collected from diseased field watermelon plants were transferred to 18 healthy plants of *D. stramonium* at the three- to four-leaf stage. Each test plant re-

ceived 30 individual thrips, and after 2 days of inoculation access the insects were killed by the pesticide carbosulfan. The test plants were further investigated for symptom development in a glasshouse where they were sprayed weekly with carbosulfan. Separate insects from the same batch of the collection were also transferred to 15 healthy plants of watermelon (*C. lanatus* 'Fubao No. 2') with 10 thrips on each. *T. palmi* from the stock colonies that were reared on healthy plants of pumpkin (*Cucurbita moschata* (Duchesne) Duchesne ex Poir.) were used as the control with the same number of insects for each plant as treatments; a total of 10 test plants of each species were used.

Thrips reared on healthy plants of *C. moschata* were also used for the transmission test. Plants of *D. stramonium* infected with a single-lesion isolate of TSWV-W and confirmed by leaf-dipping electron microscopy were used as the feeding source. Thrips at the nymph stage were transferred to the diseased plants, and the adult thrips (at least 7 days later) were picked up and transferred to a total of eight healthy plants of *D. stramonium* or *C. lanatus* 'Fubao No. 2.', each at 10 insects per plant. The inoculation access time was 2 days. Thrips feeding on healthy *D. stramonium* were used as the control.

Antiserum production. The TSWV-W isolate was purified according to the method of Gonsalves and Trujillo (6) with slight modifications. Leaves of *C. quinoa* with numerous lesions 5–7 days after inoculation were used as starting material. Fresh leaf tissue was blended in 3 vol (ml/g) of extraction buffer (0.1 M potassium phosphate buffer containing 0.01 M Na_2SO_3, pH 7.0). After being filtered through cheesecloth, the extracts were centrifuged at 4,800 *g* for 15 min. The pellets were resuspended with 1 vol (ml/g) of 0.01 M Na_2SO_3 and stirred at 4 C for 30 min, followed by clarification at 5,200 *g* for 15 min. After the supernatant was centrifuged at 52,000 *g* for 40 min, the pellets were resuspended in 0.01 M Na_2SO_3 (one-tenth of the original volume) and stirred at 4 C for 30 min. The suspension was further clarified by centrifugation at 6,500 *g* for 15 min. Aliquots of 2 ml of the concentrated virus were then layered on a 10–40% sucrose density gradient in 0.01 M Na_2SO_3 and subjected to centrifugation at 66,000 *g* for 35 min. The opalescent zones containing virus were collected, concentrated by centrifugation at 52,000 *g* for 55 min, and resuspended in 0.01 M Na_2SO_3.

For antiserum production, white New Zealand rabbits were immunized by an initial intramuscular injection with TSWV-W purified from 100 g of leaf tissue (OD_{260} 2.6–4.1), mixed 1:1 with Freund's complete adjuvant, and followed by three weekly subcutaneous injections, each with the same amount of virus mixed 1:1 with Freund's incomplete adjuvant. Antisera were collected weekly after the last injection. The titer of each bleeding was determined by a sodium dodecyl sulfate (SDS) immunodiffusion test against crude saps from TSWV-W-infected *N. benthamiana*; 5-mm wells at a distance of 5 mm were used (24).

Serological analysis. Rabbit antisera, TN-W-16 and TN-W-17, against TSWV-W produced in a current investigation were used to characterize the virus. Polyclonal antibodies against TSWV isolates from different geographical locations were also included for serological analyses. The antiserum (USA-L) against a lettuce isolate of TSWV (6) was obtained from D. Gonsalves (Cornell University, Geneva, New York). Antisera against the nucleocapsid protein of a watermelon isolate (JPN-W) or a tomato isolate of TSWV (JPN-N) of Japan (13) were provided by M. Kameya-Iwaki (University of Yamaguchi, Japan). Polyclonal antibody (GMY-I) against an amaryllis isolate of TSWV of Germany, which is serologically identical to an American impatiens isolate (16; G. Adam, *unpublished*) was provided by G. Adam (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). Monoclonal antibodies to the lettuce isolate (MAb-L) (10) and to the amaryllis isolate (MAb-I) (G. Adam, *unpublished*) from H.-T. Hsu (USDA-ARS, Beltsville, Maryland) and G. Adam, respectively, were also used in the study.

Simple indirect enzyme-linked immunosorbent assay (ELISA) (23) was used to characterize the serological reactions of TSWV-W and TSWV-NY with various polyclonal antibodies. Virus antigens in crude extracts from plants of *D. stramonium* infected with TSWV-W or TSWV-NY 10–14 days after inoculation were used for ELISA tests. Leaf tissues were ground in coating buffer (35 mM $NaHCO_3$, 15 mM Na_2CO_3, and

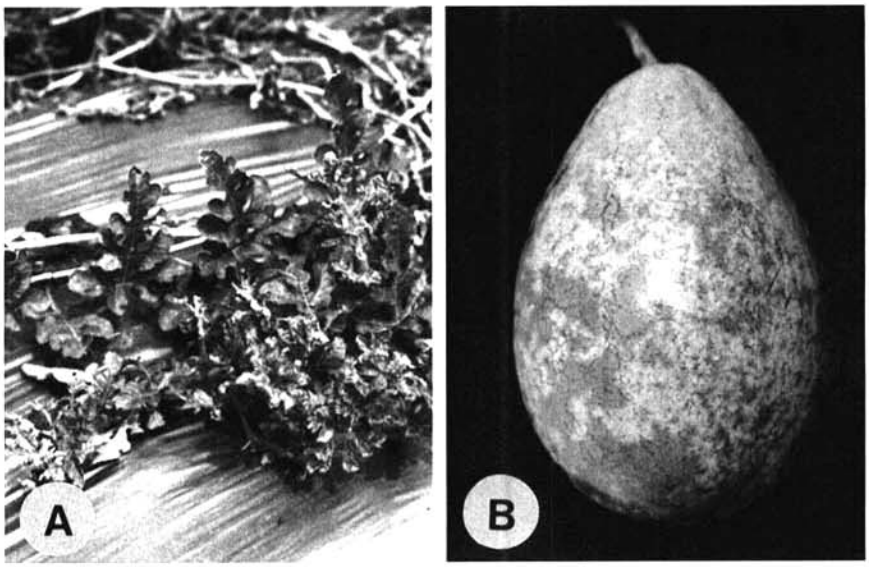

Fig. 1. (**A**) Diseased watermelon plants in the field show symptoms of stunting of tip growth, shortened internodes, leaf narrowing, and mottling. (**B**) Watermelon fruit shows symptoms of deformation, necrotic spots, and silver mottling.

Table 1. Host reactions of a tomato spotted wilt-like virus isolated from watermelon in Taiwan (TSWV-W) and an isolate of TSWV from tomato in New York (TSWV-NY)

		Reaction to TSWV-W[a]		Reaction to TSWV-NY	
Family	**Species**	**Local**	**Systemic**	**Local**	**Systemic**
Amaranthaceae	*Gomphrena globosa*	L	NS,M	L	NS,M
Chenopodiaceae	*Chenopodium amaranticolor*	L	–	L	–
	C. quinoa	L	–	L	–
Cucurbitaceae	*Citrullus lanatus*	I	Cr,NS,M	L	–
	Cucumis metuliferus	I	CS,M	L	–
	C. sativus	I	CS,M,R	L	–
Solanaceae	*Datura stramonium*	L	M,NS	L	M,NS
	Lycopersicon esculentum	I	CS,M	I	CS,M
	Nicotiana benthamiana	I	M,W	I	M,W
	N. glutinosa	L	NS,M	L	NS,M
	N. rustica	L	NS,M	L	NS,M
	Petunia hybrida	L	–	L	–

[a]Abbreviations for symptoms: CS, chlorotic spots; CR, crinkling; I, symptomless infection; L, local lesions; M, mottle; NS, necrotic spots; R, rolling of leaf edge; W, wilting; –, no infection.

0.01% NaN_3, pH 9.6). Each sample was diluted in a 10-fold series up to $1:10^6$ with coating buffer. All antisera were preadsorbed with crude saps from healthy *D. stramonium* according to the procedure of Gonsalves and Trujillo (6) and were used at a 1:1,000 dilution. Goat anti-rabbit immunoglobulin (IgG) alkaline phosphatase conjugate (Sigma No. A-8025; Sigma Chemical Co., St. Louis, MO) was used at 1 μg/ml. Enzyme-substrate reactions were conducted at room temperature and recorded by a Dynatech MR 700 ELISA reader (Dynatech Labs Inc., Chantilly, VA) at A_{405} within 60 min after the addition of the substrate.

We also used the western blotting technique to investigate the serological relationships of TSWV isolates. Leaf tissue of *N. benthamiana* or *D. stramonium* infected with TSWV was ground in 3 vol of degrading buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 3% 2-mercaptoethanol, 10% glycerol, and 0.005% bromophenol blue), boiled for 2 min, and centrifuged at 10,000 rpm for 10 min in an Eppendorf microcentrifuge. Proteins in the supernatant were separated by electrophoresis in minislab SDS gels according to the method of Laemmli (15). The separated proteins were electrophoretically transblotted to nitrocellulose (NC) paper by a Bio-Rad mini-transblot cell (Bio-Rad, Richmond, CA) at 100 V for 2 hr according to the method of Gooderham (7). After transfer, the membranes were blocked in 0.2% ovalbumin in Tris-buffered saline for 1 hr, reacted with polyclonal antibodies (1:500 dilution) or monoclonal antibodies (1:1,000 dilution) for 2 hr, and then incubated for one more hour after the addition of horseradish peroxidase labeled goat anti-rabbit IgG or goat anti-mouse IgG (both from Bio-Rad), each at a 1:4,000 dilution (0.25 μg/ml). Substrate of the horseradish peroxidase color development solution (3 mg/ml of 4-chloro-1-naphthol in cold methanol with 5 vol of 0.015% H_2O_2 in phosphate-buffered saline) was used for staining the NC paper. All reactions were at room temperature and were finally stopped by soaking the membranes in distilled water 10–30 min after the addition of the substrate.

RESULTS

Biological properties. When the diseased samples of infected watermelon plants were assayed on test plants by mechanical inoculation, development of local lesions on *C. quinoa* and systemic symptoms of mottling, wilt, and death on *N. benthamiana* were observed. A virus isolate, TSWV-W, was established by three single-lesion transfers on *C. quinoa* and then maintained in *N. benthamiana*. When healthy watermelon seedlings were inoculated with TSWV-W, they showed symptoms of plant stunting, leaf narrowing, mottling, yellow spotting, and veinal and tip necrosis; these symptoms resemble those observed on field-infected watermelon plants (Fig. 1A). When the virus from experimentally infected watermelons was transferred to *C. quinoa* and *N. benthamiana*, these hosts responded with local lesions and systemic infection, respectively, indicating that the virus recovered from field-infected watermelons is the causal agent of the disease.

TSWV-W and TSWV-NY caused similar symptoms on test plants in Amarantaceae, Chenopodiaceae, and Solanaceae (Table 1). Both viruses induced necrotic local lesions on *C. amaranticolor* Coste et Reyn., *C. quinoa*, and *P. hybrida*. They also incited similar systemic symptoms on *N. rustica* , *N. tabacum*, *N. glutinosa*, *D. stramonium*, and *Lycopersicon esculentum*. Symptoms included one or more of the following: local necrotic spots, ringspots and systemic mottling, malformation, necrosis, and wilting of apical leaves. On *Gomphrena globosa* L., both viruses caused necrotic lesions on the inoculated leaves and systemic mottle on the upper leaves. However, the lesions incited by TSWV-W appeared to be milder.

Plants in Cucurbitaceae responded differently to infection of TSWV-W and TSWV-NY (Table 1). Only local lesions developed in TSWV-NY-inoculated *C. lanatus*, *Cucumis metuliferus*, and *C. sativus*. Conversely, systemic infection occurred in TSWV-W-inoculated *C. lanatus*, *C. metuliferus*, and *C. sativus*. The TSWV-W-infected plants showed narrowing of leaf laminae, mottling, yellow spotting, malformation, and tip necrosis.

Electron microscopy. In an initial trial, PTA-stained viruslike particles were not found in extracts of naturally infected field watermelon plants. Consequently, crude saps from *N. benthamiana* inoculated with TSWV-W were prefixed with glutaraldehyde and stained with PTA. Numerous spherical particles, 75–100 nm in diameter, were present in saps of the infected plants (Fig. 2A). When crude saps from *N. rustica* and *C. lanatus* were stained with uranyl acetate, similar spherical particles were observed, but the envelope membranes were more prominent (Fig. 2B). Particle morphology of the virus was typical of the tospovirus group (17) and resembled that of TSWV-NY (not shown).

Large quasi-spherical particles with

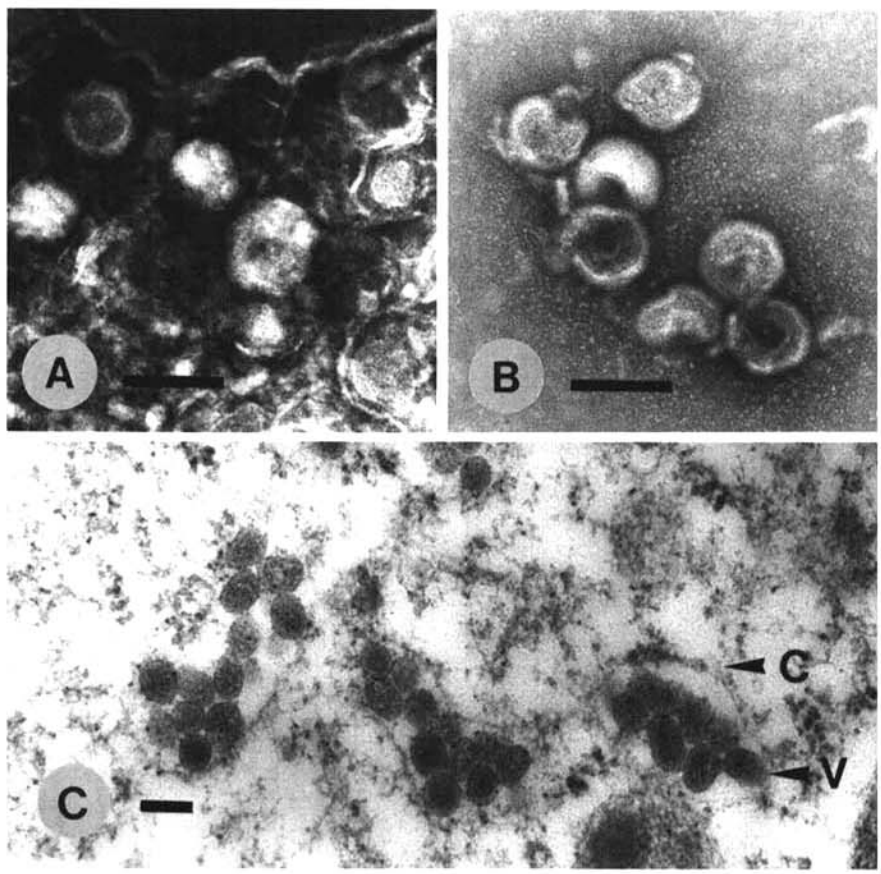

Fig. 2. Electron micrographs of tomato spotted wilt-like virus (TSWV-W) particles by leaf dipping and ultrathin sectioning. **(A)** Virus particles negatively stained by phosphotungstic acid in a dip preparation from a leaf of *Nicotiana benthamiana* infected with TSWV-W. **(B)** Virus particles negatively stained by uranyl acetate in a dip preparation from a leaf of a watermelon plant inoculated with TSWV-W. **(C)** Ultrathin sections of a leaf cell from a local lesion of *Chenopodium quinoa* inoculated with TSWV-W. Typical TSWV particles (indicated with V) appear within the cisternae of endoplasmic reticulum (indicated with C) in the cytoplasm. The bars represent 100 nm.

membranes, 80–95 nm in diameter, were also observed in ultrathin sections from the local lesion tissue of *C. quinoa* infected with TSWV-W. These virus particles appeared more regular in shape and grouped in cisternae of the endoplasmic reticulum in the cytoplasm (Fig. 2C). The virus envelope membranes were more prominent than those observed by leaf dipping.

Thrips transmission. Two to three weeks after access inoculation with *T. palmi* collected from field-diseased watermelon, 16 of 18 plants of *D. stramonium* showed symptoms of leaf mottling, ringspots, distortion, and spotted necrosis that were similar to those induced by mechanical transmission of TSWV-W. Also, eight of 15 watermelon plants that received the thrips collected from the diseased watermelons developed typical symptoms associated with TSWV-W infection. The presence of TSWV-W in test plants was confirmed by the leaf-dipping electron microscopy method mentioned above. None of controlled plants of *D. stramonium* and watermelon that received the thrips from stock colonies reared on healthy pumpkin plants showed symptoms.

Six of eight plants of *D. stramonium* and three of eight plants of watermelon showed typical symptoms incited by TSWV-W, 2–3 wk after inoculation with thrips that had fed on TSWV-W-infected *D. stramonium*. Again, the presence of membrane-bound spherical particles was confirmed by leaf-dipping electron microscopy. None of the plants of *D. stramonium* or watermelon that received the insects fed on healthy *D. stramonium* showed symptoms.

Serological assay by ELISA. Rabbit antisera TN-W-16 and TN-W-17 produced against TSWV-W had a titer of 1–2 when tested against the crude sap from diseased *N. benthamiana* in immunodiffusion tests. A strong reaction with the saps from healthy plants made them difficult to use in immunodiffusion tests. However, when preadsorbed with crude saps prepared from healthy plants, these antisera were useful for ELISA tests. In simple indirect ELISA tests, antisera TN-W-16 and TN-W-17 reacted with homologous TSWV-W, but did not react with TSWV-NY (Fig. 3A,B).

Antiserum JPN-W against the nucleocapsid protein of a watermelon isolate of Japan (13) reacted well with TSWV-W, but the reaction with TSWV-NY was not significantly higher than that of the healthy control (Fig. 3C). The reaction patterns of antiserum JPN-W were similar to TN-W-16 and TN-W-17.

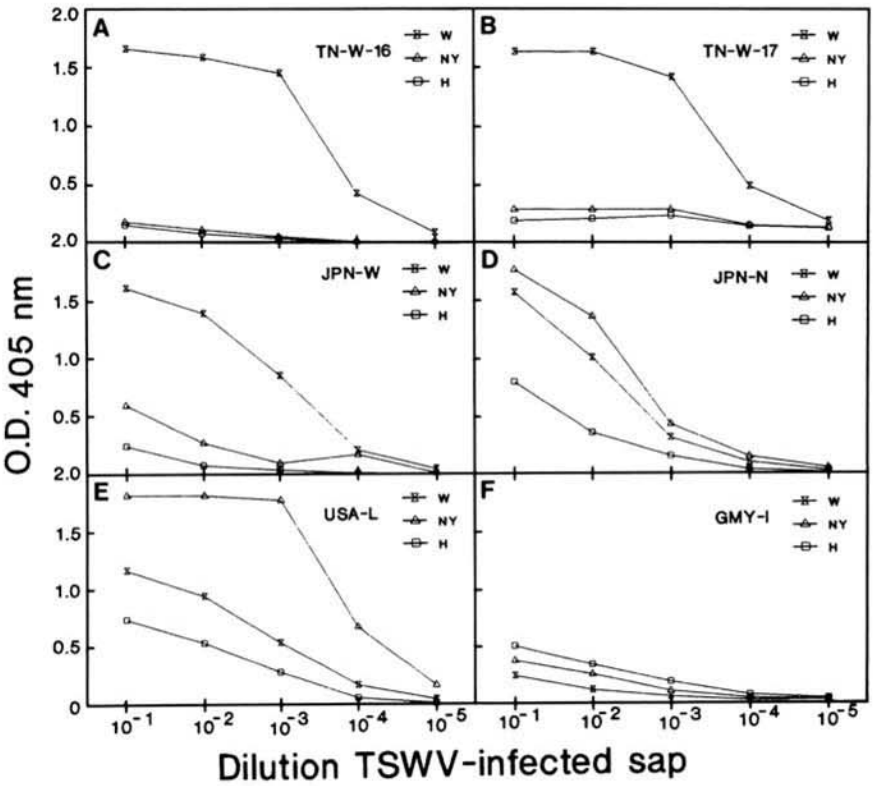

Fig. 3. Enzyme-linked immunosorbent assay determination of serological relationships of a tomato spotted wilt-like virus isolated from watermelon in Taiwan (TSWV-W) and an isolate of TSWV from tomato in New York (TSWV-NY) to various polyclonal antibodies against different isolates of TSWV. The antigens were extracted from virus-infected plants of *Datura stramonium* and diluted by 10-fold series with coating buffer. Antisera were preadsorbed with crude sap prepared from healthy *D. stramonium* and used at a 1:1,000 dilution. Alkaline phosphatase labeled goat anti-rabbit immunoglobulin (IgG) was used at a 1:1,000 dilution (1 μg/ml). Results were read 30 min after the addition of the substrate. Each reading represents an average of four duplicate wells from the plates. The following antisera were used: (**A**) and (**B**) TN-W-16 and TN-W-17, respectively, both against a watermelon isolate from Taiwan; (**C**) JPN-W against the nucleocapsid protein of a watermelon isolate from Japan; (**D**) JPN-N against the nucleocapsid protein of a tomato isolate from Japan; (**E**) USA-L against a lettuce isolate from the United States; (**F**) GMY-I against an amaryllis isolate from Germany.

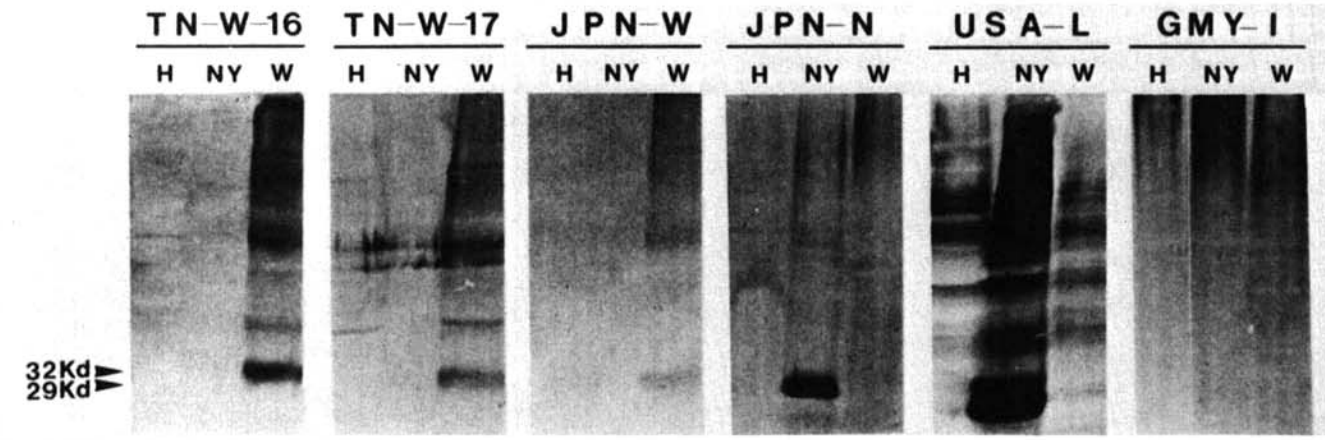

Fig. 4. Western blot analysis of serological relationships of a tomato spotted wilt-like virus isolated from watermelon in Taiwan (TSWV-W) and an isolate of TSWV from tomato in New York (TSWV-NY) to various polyclonal antibodies. Antisera used were the same as in Figure 3. H, NY, and W indicate the total proteins from healthy plants of *Nicotiana benthamiana* and plants infected with TSWV-NY and TSWV-W, respectively. Arrows at 32 kDa and 29 kDa indicate positions of nucleocapsid proteins of TSWV-W and TSWV-NY, respectively.

Antiserum JPN-N against the nucleocapsid protein of a tomato isolate from Japan (13) reacted positively with TSWV-NY. Because the nonspecific readings were quite high and reaction readings with TSWV-W were generally higher than the healthy background but less than two times those of the healthy control, it was difficult to conclude that the reaction with TSWV-W was positive (Fig. 3D).

Antiserum USA-L to a lettuce isolate of TSWV (6) reacted strongly with TSWV-NY but had high background with the healthy control. The readings of TSWV-W did not exceed two times those of the healthy control and were not considered significantly positive (Fig. 3E).

Antiserum GMY-I to the amaryllis isolate of TSWV did not react with TSWV-W or with TSWV-NY, and its nonspecific background was low (Fig. 3F).

Serological assays by western blotting. Most of the TSWV polyclonal antisera used for indirect ELISA had high nonspecific reactions with the healthy control. Thus, we conducted western blotting to clarify the ELISA results. The blotted patterns with different antisera are summarized in Figure 4. When antisera TN-W-16 was used, it strongly reacted with TSWV-W but did not react with TSWV-NY, and background reactions with the healthy control were not noticed. A distinct reaction with a 32-kDa protein, which is considered the nucleocapsid protein of the virus, was noticed. The other viral proteins were also present but not in distinct bands. Reaction patterns of antiserum TN-W-17 were similar to those of TN-W-16, except that there were some nonspecific reactions with the healthy control.

Antiserum JPN-W reacted only with the 32-kDa protein of TSWV-W but did not react with TSWV-NY and essentially had no background with the healthy control. Antiserum JPN-N reacted only with the 29-kDa nucleocapsid of TSWV-NY. This antiserum did not react to TSWV-W, and the nonspecific background was low.

Antiserum USA-L reacted strongly with TSWV-NY. A strong reaction of the 29-kDa nucleocapsid protein with this antiserum was observed. A distinct 27-kDa protein below the 29-kDa protein was also prominent and may represent the degraded form of the 29-kDa protein. Reactions of the antiserum with some other viral proteins were also noticed but were not easy to distinguish because of strong reactions of the antiserum with normal plant proteins. USA-L did not react with the nucleocapsid protein of TSWV-W.

Neither TSWV-W nor TSWV-NY reacted with GMY-I, which was produced to an amaryllis isolate serologically identical to the impatiens isolate of TSWV. The GMY-I antiserum did not react to plant proteins in western blot analysis.

Monoclonal antibody MAb-L specifically reacted to the 29-kDa nucleocapsid protein of TSWV-NY but did not react with TSWV-W at all. No reactions were observed when MAb-I was used to detect TSWV-W or TSWV-NY in western blotting (Fig. 5).

The TSWV-NY isolate used in this study did not react to antisera TN-W-16, TN-W-17, JPN-W, GMY-I, and Mab-I but reacted strongly with antisera JPN-N, USA-L, and MAb-L. The host reactions and the serological reactions clearly showed that TSWV-NY is a typical TSWV of the common L-serotype and not serologically related to our watermelon isolate.

DISCUSSION

The TSWV group, recently reclassified as a plant virus genus tospovirus of the Bunyaviridae (17), has a wide host range and causes serious diseases on a number of vegetable and ornamental species grown in warm as well as temperate regions of the world (2,3,11). Its unique particle morphology (5) and vector relationship (19) easily distinguish this virus group from all the other plant viruses. The enveloped virions are quasi-spherical (75–90 nm in diameter) containing three structural proteins of G1, G2 (glycoproteins), and N (nucleocapsid protein), and three genomic RNAs of L, M, and S (18,21,22). Current studies, including isolation, back inoculation, host reactions, electron microscopy, thrips transmission, and serological analyses by ELISA and western blotting, show that the virus isolated from diseased watermelon is an isolate of the tospovirus group. Our results also indicate that TSWV-W is a distinct isolate, which is different from the lettuce isolate and impatiens isolate (6,16).

With the exception of a watermelon isolate that incites the silver mottle disease in Okinawa (Japan) (12), most TSWV isolates reported in the literature induce only local infection on Cucurbitaceae (11,19). The TSWV-W isolated from Taiwan systemically infects watermelon and other cucurbits. Recently, devastating epidemics caused by this virus occurred in melons (*Cucumis melo*) in central and southern Taiwan. In this crop, it incited severe foliar mottling, plant stunting, upright growth of branches, and tip blight (S.-D. Yeh, *unpublished*). TSWV-W appears to be widely spread throughout the Taiwan island now and is considered one of the major limiting factors for the production of melons and watermelons.

Our results with insect transmission clearly indicated that TSWV-W is vectored by *T. palmi*, which is the most prevalent thrips on cucurbits in Taiwan. The same species also spread the silver mottle disease of watermelon in Okinawa (8). *T. palmi* has not transmitted any other TSWV isolates (19). There are, however, other thrips in Taiwan, but their role in the transmission of TSWV-W is not yet known.

Serological classification of TSWV isolates by ELISA and western blotting with polyclonal and monoclonal antibodies to virions or structural proteins has been reported, and distinct serotypes or serogroups have been recognized (1,4,16,20,23). Most of the TSWV isolates reported in the literature belong to the "common serotype" (or L-serotype) (1,4,6,23). In 1990, a different serotype was reported by Law and Moyer (16). The nucleocapsid protein of the virus isolated from impatiens was not serologically related to the common serotype and was designated I-serotype. In this investigation, TSWV-W was shown to be only related to the watermelon isolate from Okinawa (12). No serological relationship to the common L-serotype was evident (6). Although TSWV-W reacted weakly in ELISA with an antiserum to a JPN-N isolate, further analysis with western blotting clearly revealed that the nucleocapsid protein of TSWV-W is not related to the JPN-N isolate. The results

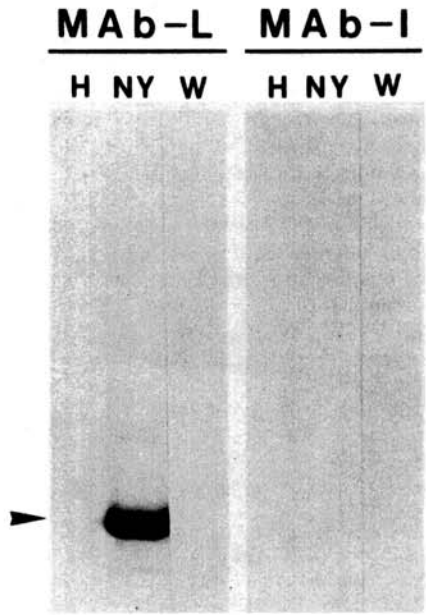

Fig. 5. Western blot analysis of serological relationships of a tomato spotted wilt-like virus isolated from watermelon in Taiwan (TSWV-W) and an isolate of TSWV from tomato in New York (TSWV-NY) to monoclonal antibodies of different serotypes of TSWV. The following antisera were used: MAb-L, monoclonal antibodies against a lettuce isolate from the United States; MAb-I, monoclonal antibody against an amaryllis isolate from Germany. H, NY, and W indicate the total protein from healthy plants of *Datura stramonium* and plants infected with TSWV-NY and TSWV-W, respectively. Position of the nucleocapsid protein of TSWV-NY is indicated by an arrow.

that TSWV-W did not react with polyclonal and monoclonal antibodies to the amaryllis isolate of TSWV indicate that the virus is a distinct isolate from the I-serotype. Unfortunately, we were not able to obtain a number of isolates from different geographical origins to provide homologous or heterologous antigens to the antisera tested. Our conclusion based on the results of Figures 4 and 5 may not be convincing because of the lack of these controls. However, for comparing the amaryllis isolate directly, we sent our watermelon isolate and antisera to G. Adam, who provided GMY-I and MAb-I antisera for this investigation. Tests done in Germany also showed that TSWV-W is serologically different from a number of isolates of the L-serotype or I-serotype in the German Collection of Microorganisms (G. Adam, *personal communication*).

We emphasize that our serological conclusions were mainly based on the relationship of the nucleocapsid protein. The major viral protein of TSWV-W that reacted in western blotting was the 32-kDa nucleocapsid protein, which is a bit larger than the 29-kDa nucleocapsid protein of TSWV-NY. A certain amount of background was evident for all polyclonal antisera used in current studies even after preadsorption with crude saps prepared from healthy plants. This is a common problem when polyclonal antibodies against partially purified TSWV were used in serological studies (1,4,23). The specific reaction patterns in western blotting are easily distinguished from the healthy controls and help clarify the uncertainty that resulted from ELISA. The reactions of glycoproteins of TSWV-W and TSWV-NY were not clear enough to distinguish them from the background in western blotting. However, the low response of JPN-N and USA-L to TSWV-W in ELISA tests (Fig. 3D,E) suggests that the viral glycoproteins may react with these antisera. Further analysis with polyclonal antibodies against purified antigens may clarify the ambiguity.

In general, TSWV-W is different from most other TSWV isolates in host reactions, vector relationship, and serology. Although TSWV-W has many properties similar to the JPN-W isolate, the identities of the two viruses and their taxonomic relationships within the tospovirus group can only be determined by further analysis of structural and nonstructural proteins and elucidation of nucleotide sequences of the genomic RNAs of the virus.

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