Eur J Plant Pathol (2011) 130:205–214
DOI 10.1007/s10658-011-9746-x

Emerging threat of thrips-borne Melon yellow spot virus on melon and watermelon in Taiwan

Jui-Chu Peng · Shyi-Dong Yeh · Li-Hsin Huang · Ju-Ting Li · Yuan-Fu Cheng · Tsung-Chi Chen

Accepted: 10 January 2011 / Published online: 14 February 2011

Abstract The thrips-borne Melon yellow spot virus (MYSV) has recently been found infecting cucurbits in Taiwan. However, this virus was indistinguishable from another thrips-borne virus species *Watermelon silver mottle virus* (WSMoV), which has been devastating on cucurbits in Taiwan for decades, when the antisera against their nucleocapsid proteins (NPs) were used for diagnosis. To understand the incidences of WSMoV and MYSV in melon and watermelon fields, a survey was conducted in central and southern Taiwan from July 2007 to December 2009. The samples collected from symptomatic plants were tested by indirect enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies (MAbs) specific to the NP of WSMoV or MYSV and the reliability of the results was verified by reverse transcription-polymerase chain reaction (RT-PCR) using species-specific primers. Among a total of 10,480 melon samples collected, 6% and 18.2% of them were found singly infected with WSMoV and MYSV, respectively, and 0.16% infected with both viruses. On the other hand, among 1,811 watermelon samples assayed, 22.4% and 9.2% samples were singly infected with WSMoV and MYSV, respectively, and 0.17% were infected with both viruses. In addition, the aphid-borne viruses *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* watermelon type (PRSV-W) and *Cucumber mosaic virus* (CMV) were also detected as prevalent viruses. Our results indicated that mixed infection with the two thrips-borne viruses is rare. Moreover, host preference for both viruses is different; WSMoV prevails on watermelon whereas MYSV is more widespread on melon. We conclude that MYSV has become a serious threat for watermelon and melon production in Taiwan and the possible control measures are discussed.

Keywords Thrips-borne tospoviruses · Cucurbit · Serology · Detection

J.-C. Peng · S.-D. Yeh (✉)
Department of Plant Pathology,
National Chung Hsing University,
Taichung 402, Taiwan
e-mail: sdyeh@nchu.edu.tw

J.-C. Peng
Division of Crop Environment, Tainan Distinct Agricultural Research and Extension Station,
Hsinhua, Tainan 712, Taiwan

L.-H. Huang
Division of Pesticide Application, Taiwan Agricultural Chemicals and Toxic Substances Research Institute,
Wufeng, Taichung 41358, Taiwan

J.-T. Li · Y.-F. Cheng · T.-C. Chen (✉)
Department of Biotechnology, Asia University,
Wufeng, Taichung 41354, Taiwan
e-mail: kikichenwolf@hotmail.com

Introduction

Melon (*Cucumis melo* L.) and watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] are two high-

value cucurbit crops and occupy cropping areas of more than 5,000 ha and 13,000 ha in Taiwan, respectively. Due to the adequate environmental conditions, over 95% and 80% of melon and watermelon, respectively, are planted in central and southern Taiwan. Plant viruses are the major pathogens for limiting production of cucurbits. Several viruses infecting cucurbits have been reported in Taiwan, such as *Cucurbit aphid-borne yellows virus* (CABYV) (Deng et al. 1997), *Cucumber green mottle mosaic virus* (CGMMV) (Hseu et al. 1987), *Cucumber mosaic virus* (CMV) (Hseu et al. 1987), *Squash leaf curl virus* (SqLCV) (Liao et al. 2007), *Papaya ringspot virus* watermelon type (PRSV-W) (Hseu et al. 1987), *Zucchini yellow mosaic virus* (ZYMV) (Hseu et al. 1985), *Watermelon silver mottle virus* (WSMoV) (Yeh et al. 1992), Melon yellow spot virus (MYSV) (Chen et al. 2008) and Cucurbit chlorotic yellows virus (CCYV) (Huang et al. 2010). Among them, aphid-borne ZYMV, PRSV-W and CMV, and the thrips-borne WSMoV have been regarded as the major viruses damaging cucurbits in Taiwan, and thrips-borne MYSV and whitefly-borne CCYV have just been found recently.

In December 2006, more than 500 ha of melon plantings were seriously damaged by virus infection, resulting in the loss of US $8 million in Tainan County. When the antiserum against the nucleocapsid protein (NP) of WSMoV was used in survey by indirect enzyme-linked immunosorbent assay (ELISA), it was suggested that the causal agent of the disaster was WSMoV. In June of the same year, the causal agent for a diseased watermelon plant originally diagnosed as WSMoV infection using WSMoV NP antiserum, was later identified as an isolate of MYSV by its different host reactions, lack of serological reaction with the monoclonal antibody (MAb) to WSMoV NP (Lin et al. 2005) and sequence analyses of its N and NSs genes (Chen et al. 2008), representing the first report for the occurrence of MYSV in Taiwan.

Both WSMoV and MYSV belong to the genus *Tospovirus* (Fauquet et al. 2005). These two tospoviruses are transmitted by the same insect vector *Thrips palmi* Karny in a persistent manner (Chen et al. 1990; Kato et al. 1999) and affect similar crops such as watermelon, wax gourd, netted melon and cucumber (Chiemsombat et al. 2008; Okuda et al. 2002; Yeh et al. 1992). They also share similar geographic distribution, mainly in the eastern and southeastern areas of Asia, including Taiwan, Japan and Thailand, and cause severe agricultural losses (Chiemsombat et al. 2008; Jan et al. 2003). Species of the genus *Tospovirus* are delineated according to a threshold of 90% amino acid (aa) identity in NP (Goldbach and Kuo 1996) and they are further classified into a serogroup or serotype based on the serological relationship of NPs (Adam et al. 1993). MYSV was originally regarded as not serologically related to WSMoV (Kato et al. 2000). However, its serological relationships with WSMoV in both NP and NSs protein have been recently verified, and the threat of MYSV in cucurbits cultivation in Taiwan has been the subject of alerts in recent years (Chen et al. 2010). Because the antiserum against the NP of WSMoV was previously used for open field detection, all positive samples were regarded as WSMoV infection and the possible cross reactions with MYSV were ignored. Thus, the incidence of MYSV infecting cucurbits in Taiwan needs accurate diagnostic tools. The MAb specific to the NP of WSMoV produced before (Lin et al. 2005) or specific to the NP of MYSV developed recently (Chen et al. 2010) can be used to identify these two tospoviruses in diseased cucurbit samples, as precisely as the results of reverse transcription-polymerase chain reaction (RT-PCR) using species-specific primer pairs (Chen et al. 2010).

To understand the disease incidences of the two thrips-borne tospoviruses that threaten the production of watermelon and melon in Taiwan, a large-scale field survey was conducted in central and southern Taiwan beginning from 2007. Indirect enzyme-linked immunosorbent assay (ELISA) using individual MAbs was performed to detect WSMoV and MYSV in the melon and watermelon samples collected from the symptomatic plants. To confirm the reliability of the serological assay, RT-PCR using species-specific primer pairs was also conducted to validate the ELISA results.

Our results indicated that the two thrips-borne tospoviruses occurred rampantly from 2007 to 2008, and they were less prevalent in 2009. Also, WSMoV is more prevailing on watermelon and MYSV more widespread on melon. On both cucurbits, mixed infection with WSMoV and MYSV is rare. In addition, aphid-borne ZYMV, PRSV-W and CMV were also detected as prevalent viruses in this investigation. The emerging threat of MYSV on watermelon and melon and the possible control measures for this virus are discussed.

Materials and methods

Sample collection

This survey was conducted during July 2007 to December 2009. Tissues from melon (*Cucumis melo* L.) and watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] plants with systemic symptoms of virus-like infection, including mosaic, chlorosis, chlorotic spots and necrotic spots etc., were collected from the cultivated areas in central and southern Taiwan. Based on the locations, townships of Yunlin, Changhua and Taichung Counties are referred to central Taiwan, and townships of Chiayi, Tainan, Kaohsiung and Pingtung Counties and districts of Tainan City are regarded as southern Taiwan. Leaf extracts from uninfected plants of melon, *Chenopodium quinoa* Willd. and *Nicotiana benthamiana* Domin. were used as negative controls. Crude extracts from plants of *N. benthamiana* infected with WSMoV, MYSV or CMV and plants of zucchini squash (*Cucumis pepo* L.) infected with ZYMV or PRSV-W were used as positive controls.

Detection of thrips-borne and aphid-borne viruses by indirect ELISA

The field-collected samples were individually tested in two replicates. Indirect ELISA was conducted following the described method (Yeh and Gonsalves 1984) with modifications. Symptomatic tissues of samples were ground in coating buffer (50 mM sodium carbonate, pH 9.6, containing 0.01% sodium azide) at a 1:40 dilution and crude extracts were used as coating antigens. The MAb specific to WSMoV NP (Lin et al. 2005) or MYSV NP (Chen et al. 2010) were diluted (10^5 or 10^4, respectively) in conjugate buffer (PBS containing 0.05% Tween 20, 2% polyvinylpyrrolidone-40 and 0.2% ovalbumin). The rabbit antiserum specific to ZYMV (Lin et al. 1998), PRSV-W (Yeh and Gonsalves 1984) or CMV (Hseu et al. 1987) was diluted in the same buffer and all were used at a 10^3 dilution. The alkaline phosphatase-conjugated goat anti-mouse or rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was used at a 1:5000 dilution as the secondary antibody. The incubation times for the reaction of crude antigens with the primary antibody and the reaction of mouse or rabbit IgG with the secondary antibody were both set at 37°C for 60 min. The absorbance at 405 nm (A_{405}) was recorded using the Victor 1420 Multilabel Counter (PerkinElmer Life Sciences, Waltham, MA) 60 to 120 min after the addition of ρ-nitrophenyl phosphate substrate (Sigma-Aldrich, St. Louis, MO) dissolved in substrate buffer (9.7% diethanolamine and 0.02% sodium azide, pH 9.8). The threshold of a positive reaction was set at two-fold readings as compared to that of the negative controls.

Verification of thrips-borne tospoviruses presence by RT-PCR

To confirm the ELISA results, RT-PCR using MYSV-specific primers MYSV-N-f (5′-GCCATGGCAT GCATGTCTACCGTTACTAAGCTGACA-3′) and MYSV-N-r (5′-GTCTAGAGGTACCAACTTCAAT GGACTTAGCTCTGGA-3′), designed from the N gene of MYSV-TW, and WSMoV-specific primers WN2963 (5′-AATAATCGGTGCCAGTCCCCTT-3′) and WN3469c (5′-ATGTCTAACGTTAAGCAGC TCACA-3′), designed from the N gene of WSMoV (Chen et al. 2010), were conducted. Total RNAs were extracted from 10% of randomly selected plant samples using the Plant Total RNA Miniprep Purification Kit (Hopegen, Taichung, Taiwan) and RT-PCR was conducted by One-Step RT-PCR Kit (Hopegen) as per 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 s, 58°C for 30 s and 72°C for 1 min.

Analysis of MYSV presence in melon fruits

Total RNAs extracted from the peduncle, exocarp, endocarp, fruit flesh and columella of the abnormal fruits collected from MYSV-infected melon plants were also assayed by RT-PCR using MYSV N gene-specific primer pair MYSV-N-f/MYSV-N-r (Chen et al. 2010) as described above.

Results

Occurrence of WSMoV and MYSV infected melon plants in central and southern Taiwan

Results of the 3-year field survey for detection of the two tospoviruses in melon are shown in Table 1.

Table 1 Field survey for plants of melon (*Cucumis melo* L.) infected with *Watermelon silver mottle virus* (WSMoV) or/and Melon yellow spot virus (MYSV) in cultivated areas of central and southern Taiwan during 2007–2009

Planting area	Year	No. of samples tested	ELISA positive					
			WSMoV		MYSV		Mixed infection	
			No.	Percentage (%)[a]	No.	Percentage (%)	No.	Percentage (%)
Central	2007	363	28	7.7	93	25.6	0	0
	2008	1,652	27	1.6	368	22.3	0	0
	2009	3,138	34	1.1	385	12.3	4	0.1
	Sum	5,153	89	1.7	846	16.4	4	0.08
Southern	2007	1,173	244	20.8	219	18.7	0	0
	2008	2,342	219	9.4	586	25.0	0	0
	2009	1,812	79	4.4	255	14.1	13	0.72
	Sum	5,327	542	10.2	1,060	19.9	13	0.24
Total		10,480	631	6.0	1,906	18.2	17	0.16

[a] ELISA-positive samples/total numbers of samples tested

Among a total of 5,153 melon samples collected from central Taiwan, 89 (1.7%) and 846 (16.4%) samples were found singly infected with WSMoV and MYSV, respectively, and only 4 (0.08%) were mixed infections with both WSMoV and MYSV. In southern areas, a total of 5,327 melon samples were tested, 542 (4.4%) and 1,060 (14.1%) samples were found infected with WSMoV and MYSV, respectively, 13 (0.24%) were mixed infections with WSMoV and MYSV. Taking central and southern areas together, a total of 10,480 symptomatic melon samples from the main planted-areas in central and southern Taiwan were collected. Among them, 631 (6.0%) and 1,906 (18.2%) samples were singly infected with WSMoV and MYSV, respectively, and only 17 (0.16%) were mixed infections with both WSMoV and MYSV. Our results indicated that in central and southern areas, the incidence of MYSV was 9.6 and 3.2 times, respectively, higher than that of WSMoV, and mixed infection with WSMoV and MYSV was rarely detected. Ten percent of total collected samples were randomly selected for RT-PCR tests to verify the reliability of ELISA results. A 0.5-kb DNA fragment corresponding to the N gene of WSMoV was amplified from the WSMoV-positive samples, and a 0.85-kb DNA fragment corresponding to the N gene of MYSV was amplified from the MYSV-positive samples. No amplicons were obtained from the WSMoV- and MYSV-negative samples using the corresponding primer pairs in RT-PCR (data not shown).

Occurrence of WSMoV and MYSV infecting watermelon in central and southern Taiwan

For watermelon, the disease incidences caused by the two tospoviruses during the 3-year field survey are shown in Table 2. Among 934 watermelon samples collected from central Taiwan, 213 (22.8%) and 120 (12.8%) samples were found singly infected with WSMoV and MYSV, respectively, and no mixed infection was detected. Among 877 watermelon samples collected from southern Taiwan, 193 (22.0%) and 46 (5.2%) samples were found infected with WSMoV and MYSV, respectively, and three (0.3%) were mixed infections with WSMoV and MYSV. All together, a total of 1,811 symptomatic watermelon specimens were collected from central and southern areas of Taiwan. Among them, 406 (22.4%) and 166 (9.2%) samples infected with WSMoV and MYSV, respectively, were detected. Moreover, only three (0.17%) samples were found mixed infection with WSMoV and MYSV. Our results indicated that in central and southern areas, the incidence of WSMoV was 1.8 and 4.3 times, respectively, higher than that of MYSV, and mixed infection with the two viruses was rare. As described above, 10% of total collected samples were randomly selected for RT-PCR tests. A 0.5-kb DNA fragment was amplified from the WSMoV-positive samples, and a 0.85-kb DNA fragment was amplified from the

Table 2 Field survey for plants of watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] infected with *Watermelon silver mottle virus* (WSMoV) or/and Melon yellow spot virus (MYSV) in planting areas of central and southern Taiwan during 2007–2009

Planting area	Year	No. of samples tested	ELISA positive					
			WSMoV		MYSV		Mixed infection	
			No.	Percentage (%)[a]	No.	Percentage (%)	No.	Percentage (%)
Central	2007	189	34	18.0	49	25.9	0	0
	2008	429	130	30.3	32	7.5	0	0
	2009	316	49	15.5	39	12.3	0	0
	Sum	934	213	22.8	120	12.8	0	0
Southern	2007	163	45	27.6	21	12.9	0	0
	2008	434	83	19.1	13	3.0	1	0.2
	2009	280	65	23.2	12	4.3	2	0.7
	Sum	877	193	22.0	46	5.2	3	0.3
Total		1,811	406	22.4	166	9.2	3	0.17

[a] ELISA-positive samples/total numbers of samples tested

MYSV-positive samples. No amplicons were obtained from the ELISA-negative samples.

Occurrence of aphid-borne viruses infecting cucurbits in southern Taiwan

The 5,327 melon samples collected from southern areas of Taiwan during 2007–2009 were also investigated for the prevalence of the most important aphid-borne viruses infecting cucurbits in Taiwan, including ZYMV, PRSV-W and CMV, using their corresponding antisera. Among them, 1,781 (33.4%), 1,346 (25.3%) and 1,137 (21.3%) samples were found infected with ZYMV, PRSV-W and CMV, respectively, and 746 (14.3%) samples were found as mixed infections with two or three of the three aphid-borne viruses. Mixed infections between aphid-borne and thrips-borne viruses were also found. Fifty-seven (1.1%) and 163 (3.1%) samples infected by any of the three aphid-borne viruses mixed with WSMoV and MYSV, respectively, were noticed (Table 3).

In addition, a total of 877 watermelon samples from southern Taiwan were also tested, and 221 (25.2%), 302 (34.4%) and 150 (17.1%) of them were found infected with ZYMV, PRSV-W and CMV, respectively. One hundred and fifty-six (17.8%) mixed infections with two or three aphid-borne viruses were detected. In addition, 44 (5%) and 4 (0.5%) samples, were mixed infections with any of the aforementioned aphid-borne viruses with WSMoV and MYSV, respectively (Table 3).

Progression of total incidences of MYSV and WSMoV infecting melon and watermelon

The total disease incidences of both tospoviruses infecting melon and watermelon from southern and central Taiwan during 2007–2009 as a whole are shown in Fig. 1. Our results showed that the infestation caused by MYSV on melon progressively increased from 2007 to 2008 from 20.3% to 23.9%, accompanied by a decreased incidence of WSMoV, 17.7% to 6.2%. However, a significant decrease of MYSV infection was observed in 2009 (12.9%) as compared to 2008 (23.9%) (Fig. 1a). A low incidence of mixed infection (0.34%) of MYSV and WSMoV on melon was found only in 2009 (Fig. 1a). The percentages of melon infected with MYSV were 1.15, 3.9 and 5.6 times higher than WSMoV in 2007, 2008 and 2009, respectively.

The total incidence of WSMoV in watermelon during 2007–2009 (19.1–24.7%) was higher than that of MYSV (5.2–19.9%) (Fig. 1b). A significant decrease of MYSV infection (5.2%) was observed from the survey of 2008. No mixed infection of MYSV and WSMoV was found in 2007, and low incidences of mixed infection (from 0.12% to 0.34%) were detected in 2008–2009 (Fig. 1b). The percen-

Table 3 Field survey for melon (*Cucumis melo* L.) and watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] infected with *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* watermelon type (PRSV-W) and *Cucumber mosaic virus* (CMV) in southern Taiwan during 2007–2009

Cucurbit	Year	No. of samples tested	ELISA positive											
			ZYMV		PRSV-W		CMV		Mixed infection (two or three aphid-borne viruses)		Mixed infection (any aphid-borne virus with WSMoV)		Mixed infection (any aphid-borne virus with MYSV)	
			No.	Percentage (%)[a]	No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)
Melon	2007	1,173	323	27.5	211	17.9	382	32.6	177	15.1	2	0.2	26	2.2
	2008	2,342	839	35.8	689	29.4	451	19.3	328	14.0	21	0.9	78	3.3
	2009	1,812	619	34.2	446	24.6	304	16.8	259	14.3	34	1.9	59	3.3
	Total	5,327	1,781	33.4	1,346	25.3	1,137	21.3	764	14.3	57	1.1	163	3.1
Watermelon	2007	163	25	15.3	27	16.6	12	7.4	22	13.5	13	8.0	2	1.2
	2008	434	112	25.8	143	33.0	41	9.5	73	16.8	17	3.9	1	0.2
	2009	280	84	30.0	132	47.1	97	34.6	61	21.8	14	5.0	1	0.4
	Total	877	221	25.2	302	34.4	150	17.1	156	17.8	44	5.0	4	0.5

[a] ELISA-positive samples/total numbers of samples tested

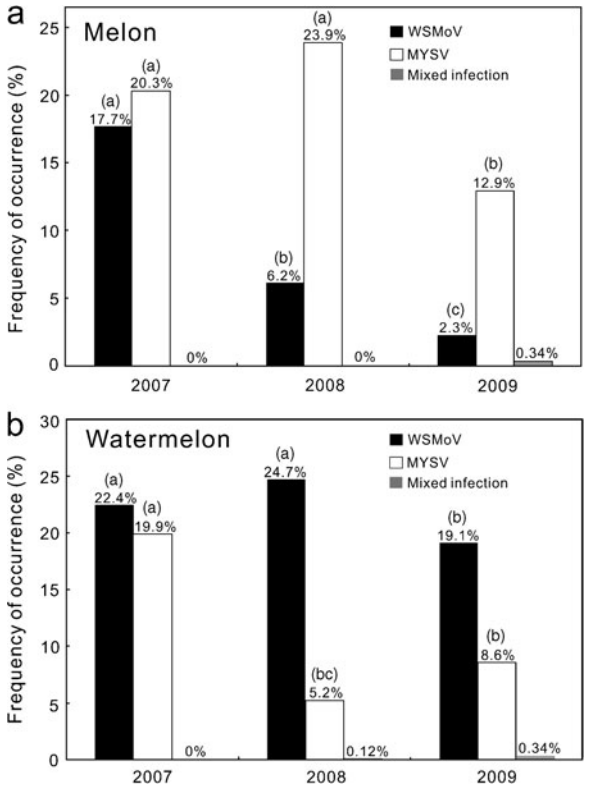

Fig. 1 Incidences (in percentages) of melon (**a**) and watermelon (**b**) infected with thrips-borne tospoviruses *Watermelon silver mottle virus* (WSMoV) or/and Melon yellow spot virus (MYSV) in central and southern Taiwan from 2007 to 2009, as detected by indirect ELISA using MAb to WSMoV NP (Lin et al. 2005) or MYSV NP (Chen et al. 2010). Percentages were calculated from the ELISA-positive numbers compared to the total numbers of the samples tested, both summarized from Tables 1 and 2. Statistical analysis in brackets was through chi-square using software JMP 5.0.1 (SAS Institute Inc.) and significance level was set at $P<0.05$

tages of watermelon infected with WSMoV were 1.13, 4.75 and 2.22 times higher than those of MYSV in 2007, 2008 and 2009, respectively.

MYSV exists in tissues of melon fruits

The fruits harvested from the MYSV-positive melon plants showed smaller, dark green and incomplete nets on surface (Fig. 2a). Using MYSV N gene-specific primer pair MYSV-N-f/MYSV-N-r, total RNAs extracted from the peduncle, exocarp, endocarp, fruit flesh and columella of the abnormal melon fruits were detected by RT-PCR for the presence of MYSV. A specific DNA fragment (0.85 kb) was amplified from each of the anatomic portions of the

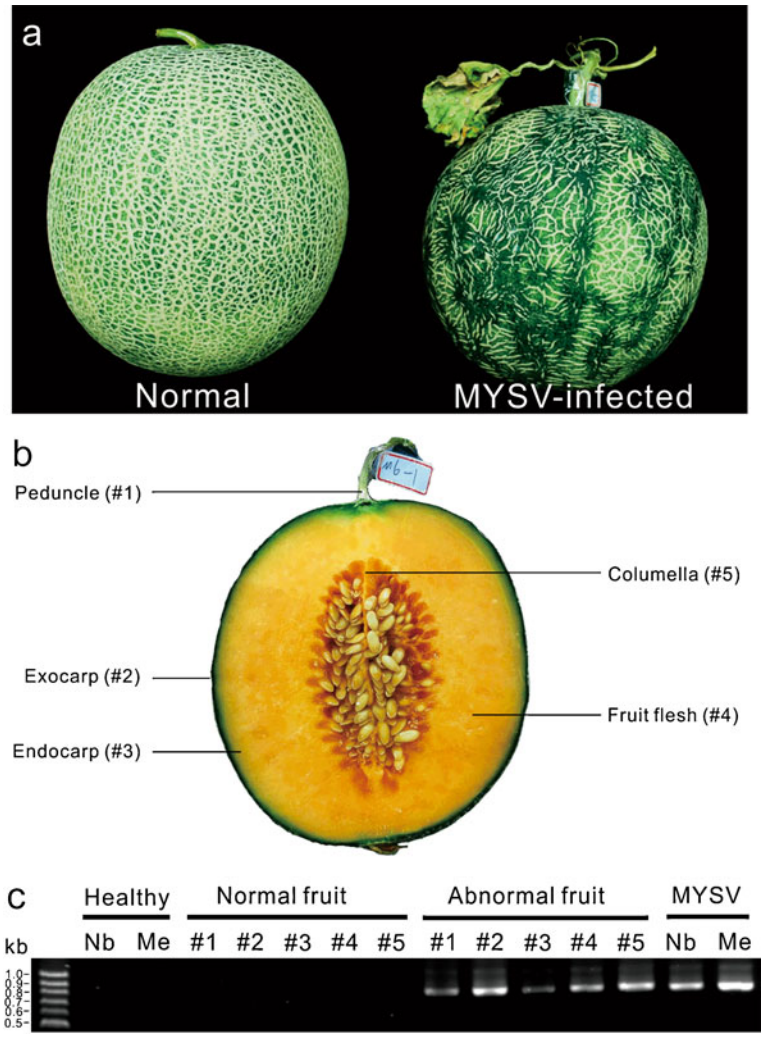

Fig. 2 Detection of Melon yellow spot virus (MYSV) in different tissues of a melon fruit cultivar Chi Gu Xiang. **a** Abnormal development of a fruit harvested from the MYSV-infected melon plant (*right*) showing reduced size and with dark green and incomplete nets, as compared with a normal fruit (*left*). **b** Longitudinal section of a melon fruit. The individual anatomic portions detected are indicated. **c** Detection of MYSV by reverse transcription-polymerase chain reaction using the MYSV-specific primer pair MYSV-N-f/MYSV-N-r. The individual portions (indicated in *numeric*) of the abnormal melon fruit were tested. Leaves of *Nicotiana benthamiana* (Nb) and melon (Me) plants infected with MYSV were used as positive controls. Leaves of a healthy plant of *N. benthamiana* or melon, and a normal melon fruit were used as negative controls

abnormal melon fruits, verifying the infection with MYSV (Fig. 2b and c).

Discussion

To assess the importance of thrips-borne tospoviruses, especially MYSV, infecting the most important cucurbit crops in Taiwan, a large field survey on melon and watermelon was conducted. Our results indicated that the occurrence, distribution, and relative incidences of WSMoV and MYSV varied from 2007 to 2009 in the cucurbit-growing regions of Taiwan. The thrips-borne tospoviruses have become increasingly important: MYSV was the major emerging threat for melon crops and WSMoV mainly infected watermelon in Taiwan. The aphid-borne viruses ZYMV, PRSV-W and CMV are still prevalent on melon and watermelon in Taiwan as previously described (Chang et al. 1987; Huang et al. 1993).

WSMoV is widely distributed around Eastern Asian countries (Iwaki et al. 1984) and is one of the major limiting factors for cucurbit production in Taiwan (Yeh et al. 1992; Yeh and Chang 1995). MYSV severely damages several cucurbitaceous crops, such as netted melon in Japan (Kato et al. 1999), watermelon, cucumber, globe luffah and wax gourd in Thailand (Chiemsombat et al. 2008) and have recently been reported occurring in watermelon in Taiwan (Chen et al. 2010). The virus was originally classified as a distinct serotype because it was considered not serologically related to any other

known tospovirus species (Kato et al. 2000). However, our recent results clearly demonstrated that MYSV is serologically related to the members of WSMoV serogroup and cannot be distinguished from WSMoV by polyclonal antibodies to WSMoV NP or MYSV NP (Chen et al. 2010). To solve this problem, the MAb specific to WSMoV NP (Lin et al. 2005) and the MAb specific to MYSV NP (Chen et al. 2010), both produced in our laboratory, were used for large-scale field surveys in this investigation. MYSV shares many similarities with WSMoV, including geographic distribution, host range, thrips vector and serological relationship (Jan et al. 2003; Chiemsombat et al. 2008; Chen et al. 2010). We observed that symptoms caused by WSMoV and MYSV on leaves of melon plants were similar under field conditions; however, they can be unambiguously identified by the two MAbs. Here we showed that the specificity of the MAbs in indirect ELISA tests for large-scale field survey was comparable with that of RT-PCR amplification as previously described (Chen et al. 2010), thus we conclude that the two MAbs are reliable diagnostic tools to distinguish MYSV from WSMoV in the field samples.

Melon plants infected by MYSV at the early planting stage developed lethal symptoms, resulting in complete loss of the crop. When melon plants were infected later, fruits could be produced. The presence of MYSV within the exocarp, endocarp, fruit flesh and columella of melon fruits from MYSV-infected plants was verified (Fig. 2). Whether MYSV invades seeds or can be seed-transmissible remains to be clarified. Our results indicated that MYSV has become a more important threat for melon production than WSMoV in Taiwan.

In melon crops, accompanied with the increased occurrence of MYSV, the incidence of WSMoV progressively decreased in Taiwan (Fig. 1a). In addition, only few cases of mixed infection by WSMoV and MYSV on melon were found. These two facts imply that the competition or cross-protection between WSMoV and MYSV exists in the same host plants and possibly in the insect vector *T. palmi*. Careful studies for mutual cross-protection effectiveness in cucurbit hosts and thrips vectors may prove this notion.

The incidences of WSMoV and MYSV infecting melon and watermelon are geographically different in Taiwan. Disease incidences of WSMoV- or MYSV-infected melon in southern Taiwan are higher than those in central Taiwan. On the contrary, disease incidences of WSMoV- and MYSV-infected watermelon were higher in central Taiwan. Over 50% melon cropping areas of Taiwan are located in Tainan County of southern Taiwan. Cucurbit crops planted in central Taiwan are mainly watermelon, wax gourd and cucumber. The different cropping ratios of melon and watermelon in the two areas apparently affect the distribution and the incidences of the two tospoviruses. On the other hand, melon plants are commonly planted in the plastic tunnels in Taiwan rather than in open fields. This is different from the cultivation of other cucurbits. The plastic tunnels provide an ideal warm and contained environment for thrips vectors, especially in the early spring. Additionally, the climate of southern areas is much warmer than central areas in Taiwan, especially in autumn and winter, maintaining the thrips vector in a higher population for overwintering. In addition to watermelon, wax gourd is also more susceptible to WSMoV than MYSV in observations from our other small-scale field surveys (Chen et al. 2010). Overall, the infestations of WSMoV and MYSV are apparently affected by the environmental factors, crop categories and agricultural practices.

Surprisingly, the incidence of MYSV in Taiwan rapidly increased in recent years, after it was discovered from watermelon in 2006. It is possible that MYSV may have occurred in Taiwan earlier but was just masked by WSMoV due to the serological cross-reaction when the antiserum to WSMoV NP was used for virus detection. In addition, several cucurbit crops can be damaged by MYSV, including netted melon (Kato et al. 1999), watermelon (Chen et al. 2008), balsam pear (Takeuchi et al. 2009), squash and cucumber (Takeuchi et al. 2001; Chiemsombat et al. 2008). These crops are also widely grown in Taiwan; whether they are also infected with MYSV remains to be further investigated.

In this investigation, melon and watermelon crops infected by the aphid-borne plant viruses, including ZYMV, PRSV-W and CMV, were also detected as the prevalent viruses in the 3-year surveys (Table 3), indicating that these aphid-borne viruses still seriously damaged cucurbit crops in Taiwan. Mixed infection of aphid-borne viruses with thrips-borne viruses may create devastating effects because of the synergism. Fortunately, the peak of

alate aphids is in the cool seasons of autumn and winter in Taiwan, and the peak of thrips population is in the warm seasons of spring and summer, thus the deadly effect of mixed infection with the two virus groups has not occurred widely.

In some cases, for instance, 106 of 5,327 melon samples (2%) in the southern area, no serological responses were found when the symptomatic cucurbit tissues incubating with the MAbs and antisera used in this study. Whether these cucurbit samples were infected with unknown viruses or other cucurbit-infecting tospoviruses, such as Watermelon bud necrosis virus (Singh and Krishnareddy 1996), *Zucchini lethal chlorosis virus* (Bezzera et al. 1999) and Melon severe mosaic virus (Ciuffo et al. 2009), remains to be clarified in future investigations. Through the support of the Council of Agriculture of Taiwan, the virus survey is still conducted weekly, and data of the continuous field survey provide important information to develop a monitoring system for disease forecasting and control.

In December 2006, more than 500 ha of melon crops were seriously damaged by MYSV infection, resulting in the loss of US $8 million in Tainan County. The combined efforts of the Bureau of Animal and Plant Health Inspection and Quarantine, Tainan Distinct Agricultural Research and Extension Station, Tainan County and City governments and Farmers' Associations of Townships were organized to carry out an emergency control measure. Growers are not allowed to raise melon or watermelon seedlings themselves under open conditions. Sanitation of nursery in a contained condition to avoid thrips invasion at the seedling stage is highly recommended. Insecticides such as Chlorfenapyr (Sinon, Taichung, Taiwan), Imidacloprid (Sinon) and Deltamethrin (Sinon) were subsidized for elimination of thrips at the early stages after transplanting and for control of the population of thrips in cultivation time. These emergent control measures were effective as reflected in the dramatically decreased incidences of both MYSV and WSMoV in melon crop in 2009.

Although tospovirus diseases of melon and watermelon can be controlled by spraying insecticides, the method creates environmental hazards. Alternatively, an RNA silencing-based transgenic approach is the most efficient strategy for control of plant virus diseases (Baulcombe 2004). Resistance mediated by RNA silencing is sequence homology-dependent and determination of the specific molecular features of the causal agents is important. Our results have offered valuable information for the epidemics of thrips-borne tospoviruses in Taiwan, clarified the emerging threats of MYSV on melon and watermelon and provided insight for the development of effective control measures.

Acknowledgments 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.

References

Adam, G., Yeh, S.-D., Reddy, D. V. R., & Green, S. K. (1993). Serological comparison of tospovirus isolates from Taiwan and India with impatiens necrotic spot virus and different tomato spotted wilt virus isolates. *Archives of Virology, 130*, 237–250.

Baulcombe, D. (2004). RNA silencing in plants. *Nature, 431*, 356–363.

Bezzera, I. C., de Resende, O., Pozzer, L., Nagata, T., Kormelink, R., & de Avila, A. C. (1999). Increase of tospoviral diversity in Brazil with the identification of two new tospovirus species, one from chrysanthemum and one from zucchini. *Phytopathology, 89*, 823–830.

Chang, Y. M., Hsaio, C. H., Yang, W. Z., Hseu, S. H., Chao, Y. J., & Huang, C. H. (1987). The occurrence and distribution of five cucurbit viruses on melon and watermelon in Taiwan. *Journal of Agricultural Research, 36*, 389–397.

Chen, C. C., Shy, J. F., & Yeh, S. D. (1990). Thrips transmission of *Tomato spotted wilt virus* from watermelon. *Plant Protection Bulletin, 32*, 331–332.

Chen, T.-C., Lu, Y.-Y., Cheng, Y.-H., Chang, C.-A., & Yeh, S.-D. (2008). *Melon yellow spot virus* in watermelon: a first record from Taiwan. *Plant Pathology, 57*, 765.

Chen, T.-C., Lu, Y.-Y., Cheng, Y.-H., Li, J.-T., Yeh, Y.-C., Kang, Y.-C., et al. (2010). Serological relationship between Melon yellow spot virus and *Watermelon silver mottle virus* and differential detection of the two viruses in cucurbits. *Archives of Virology, 155*, 1085–1095.

Chiemsombat, P., Gajanandana, O., Warin, N., Hongprayoon, R., Bhunchoth, A., & Pongsapich, P. (2008). Biological and molecular characterization of tospoviruses in Thailand. *Archives of Virology, 153*, 571–577.

Ciuffo, M., Kurowski, C., Vivoda, E., Copes, B., Masenga, V., Falk, B. W., et al. (2009). A new *Tospovirus* sp. in cucurbit crops in Mexico. *Plant Disease, 93*, 467–474.

Deng, T. C., Tsai, C. H., Chen, Y. F., Chang, C. A., Hsiao, C. H., & Lecoq, H. (1997). First report of cucurbit aphid-borne yellows luteovirus in Taiwan. *Plant Protection Bulletin, 39*, 395–396.

Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U., & Ball, L. A. (Eds.). (2005). *Virus taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier/Academic.

Goldbach, R. W., & Kuo, G. (1996). Tospoviruses and thrips. *Acta Horticulturae, 431*, 16–43.

Hseu, S. H., Wang, H. L., & Huang, C. H. (1985). Identification of zucchini mosaic virus from *Cucumis sativus*. *Journal of Agricultural Research of China, 34*, 87–95.

Hseu, S. H., Huang, C. H., Chang, C. A., Yang, W. Z., Chang, Y. M., & Hsiao, C. H. (1987). The occurrence of five viruses in six cucurbits in Taiwan. *Plant Protection Bulletin, 29*, 233–244.

Huang, C. H., Liang, S. C., Deng, T. C., & Hseu, S. H. (1993). Comparison of diagnostic hosts and serological tests for four cucurbit potyvirus. *Plant Pathology Bulletin, 2*, 169–176.

Huang, L. H., Tseng, H. H., Li, J. T., & Chen, T. C. (2010). First report of Cucurbit chlorotic yellows virus infecting cucurbits in Taiwan. *Plant Disease, 94*, 1168.

Iwaki, M., Honda, Y., Hanada, K., & Tochihara, H. (1984). Silver mottle disease of watermelon caused by tomato spotted wilt virus. *Plant Disease, 68*, 1006–1008.

Jan, F.-J., Chen, T.-C., & Yeh, S.-D. (2003). Occurrence, importance, taxonomy, and control of thrips-borne tospoviruses. In H.-C. Huang & S. N. Acharya (Eds.), *Advances in plant disease management* (pp. 391–411). Trivandrum: Research Signpost.

Kato, K., Hanada, K., & Kameya-Iwaki, M. (1999). Transmissions mode, host range and electron microscopy of a pathogen causing a new disease of melon (*Cucumis melo*) in Japan. *Annuals of the Phytopathological Society of Japan, 65*, 624–627.

Kato, K., Hanada, K., & Kameya-Iwaki, M. (2000). *Melon yellow spot virus*: a distinct species of the genus *Tospovirus* isolated from melon. *Phytopathology, 90*, 422–426.

Liao, J. Y., Hu, C. C., Lin, T. K., Chang, C. A., & Deng, T. C. (2007). Identification of *Squash leaf curl Philippines virus* on *Benincasa hispida* in Taiwan. *Plant Pathology Bulletin, 16*, 11–18.

Lin, S. S., Hou, R. F., Huang, C. H., & Yeh, S. D. (1998). Characterization of *Zucchini yellow mosaic virus* (ZYMV) isolates collected from Taiwan by host reactions, serology, and RT-PCR. *Plant Protection Bulletin, 40*, 163–176.

Lin, Y.-H., Chen, T.-C., Hsu, H.-T., Liu, F.-L., Chu, F.-H., Chen, C.-C., et al. (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.

Okuda, M., Takeuchi, S., Taba, S., Kato, K., & Hanada, K. (2002). *Melon yellow spot virus* and *Watermelon silver mottle virus*: outbreak of cucurbit infecting tospovirus in Japan. *Acta Horticulturae, 588*, 143–148.

Singh, S. J., & Krishnareddy, M. (1996). Watermelon bud necrosis: a new tospovirus disease. *Acta Horticulturae, 431*, 68–77.

Takeuchi, S., Okuda, M., Hanada, K., Kawada, Y., & Kameta-Iwaki, M. (2001). Spotted wilt disease of cucumber (*Cucumis sativus*) caused by Melon yellow spot virus. *Japanese Journal of Phytopathology, 67*, 46–51.

Takeuchi, S., Shimomoto, Y., & Ishikawa, K. (2009). First report of Melon yellow spot virus infecting balsam pear (*Momordica charantia* L.) in Japan. *Journal of General Plant Pathology, 75*, 154–156.

Yeh, S. D., & Chang, T. F. (1995). Nucleotide sequence of the N gene of watermelon silver mottle virus, a proposed new member of the genus *Tospovirus*. *Phytopathology, 85*, 58–64.

Yeh, S.-D., & Gonsalves, D. (1984). Purification and immunological analyses of cylindrical-inclusion protein induced by *Papaya ringspot virus* and *Watermelon mosaic virus* 1. *Phytopathology, 74*, 1273–1278.

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