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First record of melon yellow spot virus in pumpkin and its occurrence in cucurbitaceous crops in Thailand

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Abstract

Melon yellow spot virus (MYSV) was previously reported from wax gourd in Thailand. A survey of cantaloupe, cucumber, melon, pumpkin and watermelon plants was carried out to determine if MYSV occurred more widely in cucurbit species. The survey revealed melon was mostly infected with MYSV. In addition, MYSV was detected for the first time in pumpkin in Thailand.

Keywords Melon yellow spot virus · Cucurbitaceous · Pumpkin · ELISA · RT-PCR · TEM

Melon yellow spot virus (MYSV), belonging to the genus *Tospovirus* of the family Bunyaviridae, was first reported from infected melon (*Cucumis melo*) in Japan (Kato et al. 2000) and later found in cucurbit crops in many countries in Asia, such as watermelon in Taiwan (Chen et al. 2007), wax gourd in Thailand (Chiemsombat et al. 2008), balsam pear in Japan (Takeuchi et al. 2009) and melon in China (Gu et al. 2012). Recently, MYSV had been reported to infect cucurbits in Ecuador, South America (Quito-Avila et al. 2014). In Thailand, MYSV was mainly detected from cucurbits including cucumber, luffa, melon, watermelon and wax gourd in central and east Thailand (Chiemsombat et al. 2008). Moreover, MYSV was reported to infect pepper in southern Thailand (Sunpapao 2012) which was the first record of MYSV infecting other crops other than cucurbit plants in Thailand. However, detailed diagnosis of MYSV on cucurbit plants in northern Thailand had not been done. MYSV is transmitted by thrips (*Thrips palmi*) as a persistent propagative manner (Kato et al. 2000). Symptoms on host plants induced by MYSV include chlorotic spots, malformation, mosaic, mottle, necrotic spots and yellowing and those symptoms can be observed on both leaf and fruit which cause unmarketable products (Sugiyama et al. 2009). In this study, we detected MYSV from cucurbit leaf samples by using plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA), reverse transcription polymerase chain reaction (RT-PCR) and transmission electron microscope (TEM) and also studied the biological and molecular characterisation of MYSV.

A survey was conducted in cucurbit (cantaloupe, cucumber, melon, pumpkin and watermelon) growing areas in northern Thailand including Chiang Mai, Chiang Rai and Lamphun provinces, both in greenhouse and open field. Symptomatic leaves showing virus-like symptoms were collected (3 leaves/plant) for the detection of MYSV by PTA-ELISA using monoclonal antibody specific to MYSV (BIOTEC, Thailand) according to manufacturer's instruction. The percentage of disease incidence (PDI) was calculated as described by Ali et al. (2013). A total of 201 cucurbit leaf samples were collected and the result of the detection showed that 127 from 201 samples (63.18%) could be detected for MYSV and among these, melon was the most MYSV-infected cucurbit with 46.26%, followed by cantaloupe (13.93%), cucumber (2.48%) and pumpkin (0.49%), but not detected from watermelon (Table 1). The symptoms caused by MYSV were various and depended upon host plants (Fig. 1).

The 8 cucurbit leaf samples including cantaloupe, cucumber, melon and pumpkin, collected from different provinces, which showed positive to ELISA were used for RT-PCR

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Table 1 Number of cucurbit leaf samples collected from northern Thailand for detection of melon yellow spot virus (MYSV) by PTA-ELISA

Host	No. of samples collected	No. of samples positive	Incidence (%)
Cantaloupe	44	28	13.93
Cucumber	12	5	2.48
Melon	123	93	46.26
Pumpkin	15	1	0.49
Watermelon	7	0	0.00
Total	201	127	63.18

detection. Total RNA was extracted from leaves using TRIzol Reagent (Invitrogen, USA) and cDNA was synthesised using RevertAid First Strand synthesis kit (Invitrogen, USA). The PCR amplification was performed by PCR Master Mix 2X (Invitrogen, USA) using nucleocapsid (N) protein gene specific primers and the PCR conditions followed the method of Charlermroj et al. (2017). The PCR product was analysed by 1% agarose gel electrophoresis stained by RedSafe (iNtRON, South Korea). Expected amplicon size was approximately 840 bp.

Nucleotide sequences were directly analysed using fluorescent dye-terminator sequencing on ABI Prism™ 3730xl DNA sequencers (Applied Biosystems, Foster City, CA). All obtained sequences were analysed and aligned using BLAST and MAFFT v.7.0 (Katoh and Standley 2013), respectively, and then deposited in GenBank. Phylogenetic tree analysis of N genes was based on Maximum Likelihood (ML) method which was performed in MEGA7 (Kumar et al. 2016). The 840 nucleotides of N genes of four MYSV isolates were obtained from cantaloupe (Ca1), cucumber (Cu2), pumpkin (P2) and melon (MHK) with 99% identities among different isolates and shared 99% nucleotide with those MYSV isolates which was reported from Thailand, China and Japan (Fig. 2).

MYSV-P2 and MYSV-MHK were isolated from pumpkin and melon, respectively, by using single local isolation on *Chenopodium amaranticolo*r and were maintained in cucumber. The presence of MYSV was detected by PTA-ELISA. Other cucurbit-infecting viruses including Cucumber green mottle mosaic virus (CGMMV), Cucumber mosaic virus (CMV), Papaya ringspot virus (PRSV), Tomato yellow leaf curl virus (TYLCV), Watermelon silver mottle virus (WSMoV), Watermelon mosaic virus-2 (WMV-2) and Zucchini yellow mosaic virus (ZYMV) were also detected by using ELISA to confirm single infection of MYSV and

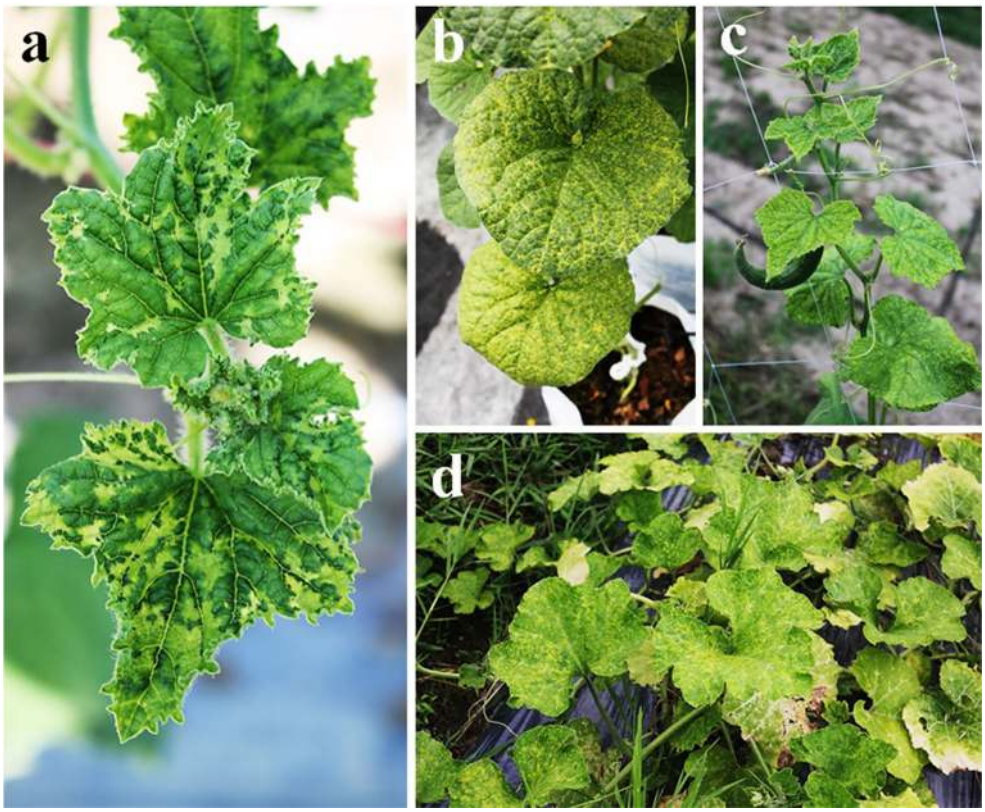

Fig. 1 Symptoms caused by Melon yellow spot virus (MYSV) on cucurbit host plants; **a** mosaic on melon young leaves, **b** numerous necrotic spots on older leaves of melon, **c** yellowing and vein banding on cucumber leaves and **d** mosaic on pumpkin leaves

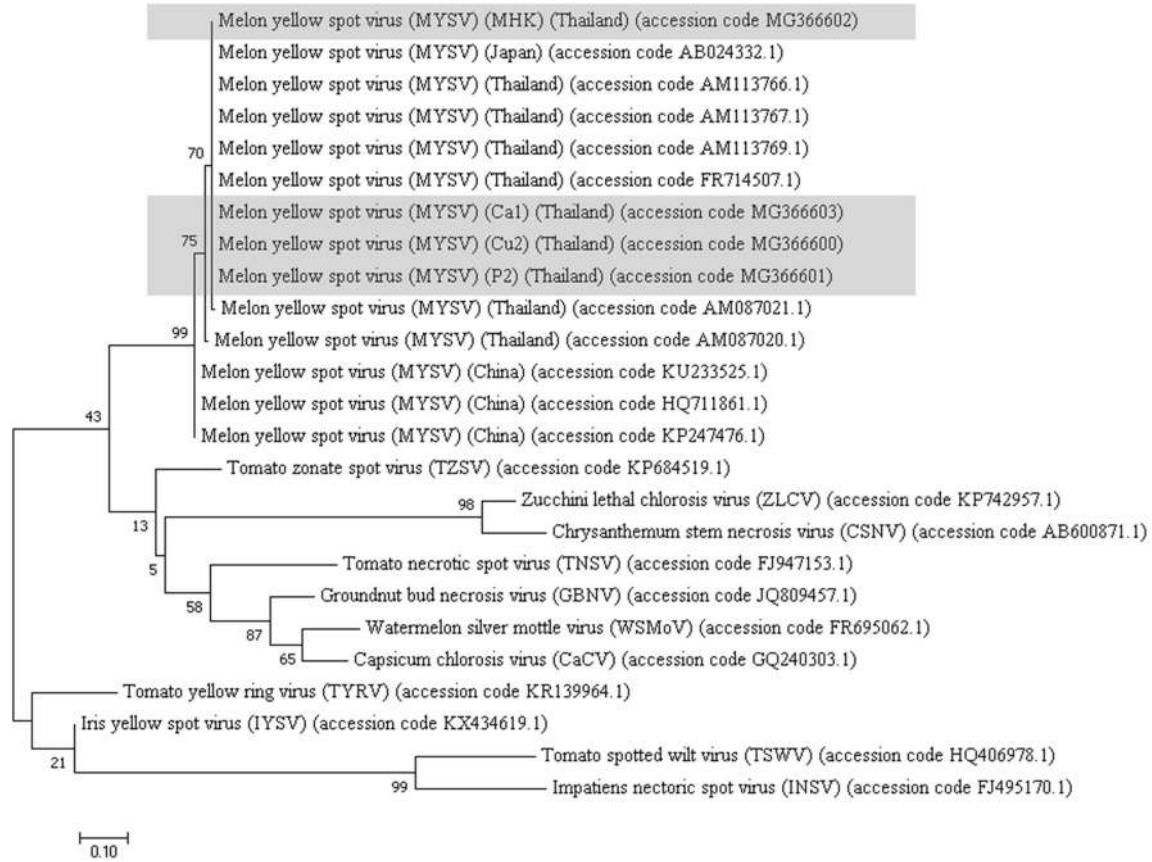

Fig. 2 Maximum likelihood phylogenetic tree of nucleocapsid (N) protein gene sequences of Melon yellow spot virus (MYSV) isolates MHK, Ca1, Cu2 and P2 (indicated by gray background) in comparison with previously-reported MYSV and other tospoviruses. Analysis was done with MEGA7 with 1000 replicates of bootstrapping

showed no positive results. The sixteen indicator plants (Table 1) were mechanically inoculated for host range testing of those two isolates. All of Cucurbitaceae consisting of melon (*Cucumis melo*), cantaloupe (*C. melo* var. *cantalupensis*), cucumber (*C. sativas*), watermelon (*Citrullus lunatas*), zucchini (*Cucurbita pepo*) and pumpkin (*Cucurbita maxima*) showed

Table 2 Symptoms induced by melon yellow spot virus (MYSV) isolate MHK and P2 on indicator plants

Family	Species	Symptom development[a]	
		Inoculated leaf	Upper leaf
Amaranthaceae	*Gomphrena globosa*	NS[b]	–
Chenopodiaceae	*Chenopodium amaranticolor*	CS	–
Cucurbitaceae	*Cucumis melo*	NS	M, Ma, NS, Y
	C. melo var. *cantalupensis*	NS	M, Ma, NS, Y
	C. sativas	NS	M, NS, VB, Y
	Citrullus lunatas	NS	M, NS
	Cucurbita pepo	NS	M, Ma
	Cucurbita maxima	NS	M, YS
Leguminosae	*Vigna unguiculata*	NS	-
	V. unguiculata ssp. *sesquipedalis*	NS	-
Solanaceae	*Capsicum annuum*	-	-
	Lycopersicon esculentum	-	-
	Nicotiana glutinosa	NS	M, Ma, NS
	N. tabacum cv. Samsun NN	-	-
	N. tabacum cv. Xanthi nc	-	-
	Petunia hybrida	-	-

[a] All plants were kept in the greenhouse at 25–28 °C

[b] *CS*, chlorotic spot; *M*, mosaic; *Ma*, malformation; *NS*, necrotic spot; *VB*, vein banding; *Y*, yellowing; *YS*, yellow spots; –, no symptom

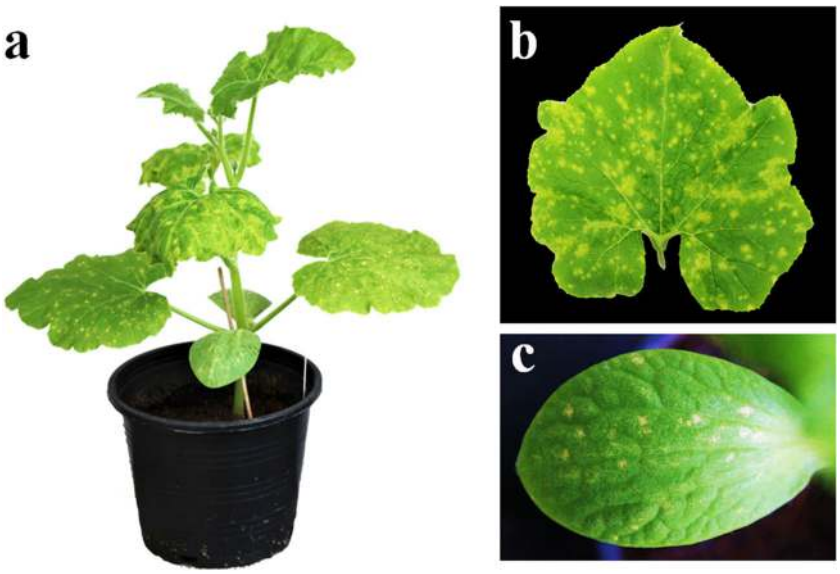

Fig. 3 Symptoms caused by Melon yellow spot virus (MYSV) on pumpkin; **a** symptomatic pumpkin plant, **b** chlorotic spots on pumpkin leaf and **c** necrotic spots on inoculated cotyledon

systemic symptoms including chlorotic or necrotic spots on inoculated leaves (within 5 days post-inoculation). Also, many symptoms were observed on uninoculated leaves, new growth leaves, (within 14 days post-inoculation), such as malformation, mosaic, mottle, vein banding and yellowing (Table 2). Pumpkin inoculated with MYSV-P2 showed mosaic and chlorotic spots on upper leaves (Fig. 3a, b) and necrotic spots were observed on inoculated cotyledons (Fig. 3c). Local symptoms were observed in *Gomphrena globosa*, *Petunia hybrida* and *Vigna unguiculata* within 10 days post-inoculation (Table 2). This result had some differences with the previous report of Chiemsombat et al. (2008) who studied MYSV-WG17 isolated from wax gourd in central Thailand. MYSV-WG17 did not cause any characteristics on primary leaves of *V. unguiculata* while MYSV-P2 induced necrotic spots. Additionally, MYSV-P2 caused no symptoms on *Capsicum annuum* and *Lycopersicon esculentum* while MYSV-WG17 was not tested. However, *Nicotiana glutinosa* showed both necrotic spots and systemic necrosis which was similar to the result of MYSV-WG17. Although their biological characteristics might be different, the genome sequences of the N gene were similar and shared 98–99% identity. These results inferred the biological variation among MYSV isolates found in different regions of Thailand. However, MYSV-P2 and MYSV-MHK which were isolated from different host plants in this study had the same biological characteristics on indicator plants.

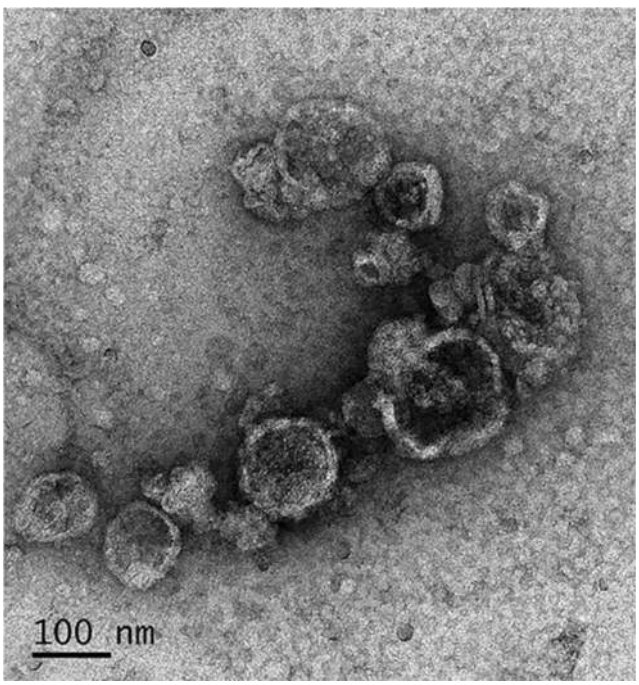

Fig. 4 Transmission electron micrograph of Melon yellow spot virus (MYSV) showing enveloped nearly-spherical particles with 80–150 nm diameter

MYSV particles in inoculated pumpkin were observed by TEM. The samples were prepared by dip preparation and negatively contrasted as described by Kumar et al. (2014) with major modifications. Leaves were ground with 0.1 M phosphate buffer (pH 7.0) in the ratio 1:0.5 (g/ml) and centrifuged at 12,000 rpm for 10 min. A formvar-coated copper grid was floated on the supernatant for 2 min and then washed with distilled water for 3 min. The grid was fixed with 1% glutaraldehyde for 5 min, stained with 2% uranyl acetate for 4 min and then observed under TEM JEM-2200FS (JOEL, USA). Enveloped nearly-spherical particles with 80–150 nm diameter of MYSV were observed (Fig. 4). This result was related to Kato et al. (2000) who reported the new virus found in melon in Japan that had enveloped particles and named Melon yellow spot virus (MYSV).

The results of this research can be used for the surveillance of MYSV in cucurbit to alert the occurrence of MYSV new

host plants. Nevertheless, squash and zucchini have no information of MYSV detection, but they have risk of infection by MYSV because they are host plants of thrips (*Thrips palmi*), a vector of MYSV (Riley et al. 2011). MYSV-P2, isolated from pumpkin, was identified by back-inoculation to indicator plants, especially pumpkin, as the basis of Koch's postulates to confirm the pathogenicity of MYSV and the analysis of N gene sequence (accession code MG366601) compared with those reported MSYV revealed this is the first report of MYSV infecting pumpkin in Thailand.

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