Eur J Plant Pathol (2014) 140:193–197
DOI 10.1007/s10658-014-0454-1

# Detection and occurrence of *melon yellow spot virus* in Ecuador: an emerging threat to cucurbit production in the region

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Accepted: 8 May 2014 / Published online: 20 May 2014


**Abstract** More than fifty viruses have been reported in cucurbit crops worldwide. In Ecuador, approximately 3,000 ha of watermelon, melon and cucumbers are cultivated annually, but there have been few studies to identify viruses responsible for epidemics. During this study, sequencing of double-stranded RNA (dsRNA) extracted from watermelon and melon leaves showing virus-like symptoms revealed the presence of *Melon yellow spot virus* (MYSV, genus *Tospovirus*) and the partially described Cucumis melo endornavirus (CmEV). Specific primers, designed to detect each virus, showed that MYSV was present in 40, 64 and 67 % of symptomatic watermelon, cucumber and melon samples, respectively. For CmEV, 95 % of both symptomatic and asymptomatic melon plants tested positive. However, the virus was not detected in watermelon or cucumber. Sequence comparisons showed nucleotide identities of 97 % and 94 % for the polymerase and the nucleocapsid protein, respectively, between the Ecuadorean MYSV and the one reported from Japan. To the best of our knowledge, this is the first report of MYSV and CmEV in Ecuador and the Americas.

**Keywords** Melon · Watermelon · Cucumber · Tospovirus · Endornavirus

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More than fifty viruses from several different taxonomic groups have been reported as disease agents in cucurbit crops (Zitter et al. 1996; Lecoq et al. 1998; Lecoq and Desbiez 2012). In Ecuador, watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) are the two most important cucurbit crops cultivated in coastal areas, where viral diseases have been attributed as the main limiting factor in terms of yield and fruit quality (Ochoa, personal communication).

During the past decade, severe disorders have emerged causing considerable losses in watermelon and melon in Manabí and Santa Elena, the main cucurbit producing provinces of Ecuador. Common symptoms include vein clearing, mosaic and leaf deformation. In watermelon, an additional symptom, commonly referred to as "foxtail", is sometimes observed when leaves at the terminal part of the vines are oriented upward.

In 2007, an ELISA-based virus survey showed that *Papaya ring spot virus* (PRSV, genus *Potyvirus*) and

*Cucumber mosaic virus* (CMV, genus *Cucumovirus*) were commonly found in melon and watermelon (Insuasti, personal communication). This finding was supported by a more recent survey conducted by Espinoza et al. (2010), where in addition to PRSV and CMV, *Squash mosaic virus* (SqMV, genus *Comovirus*) was reported in melon. The tospoviruses *Watermelon silver mottle* (WSMoV) and *Tomato spotted wilt* (TSWV) were not detected in tested samples from either crop. Although, the presence of single and mixed infections between CMV, PRSV and SqMV was frequent, their absence in some symptomatic plants suggested the existence of additional viruses (Espinoza et al. 2010).

In this study, sampling of watermelon and melon showing virus-like symptoms was conducted in different growing areas of Manabí and Santa Elena. Initial sampling was done from January (rainy season) to August (dry season) of 2013. Watermelon varieties sampled in this study included 'Glory Jumbo' and 'Royal Charleston'. For melon, sampled varieties included 'Primo' and 'Journey'. Symptoms commonly observed in the field are shown in Fig. 1.

Six 15-g batches of leaf tissue were pooled from a total of 30 symptomatic leaves per crop and used for double-stranded RNA (dsRNA) extraction following the protocol described by Morris and Dodds (1979). The dsRNA was used as template for reverse transcription (RT) using anchored-random primers and PCR-amplified using the known sequence of the primer as described (Froussard 1992). Randomly amplified

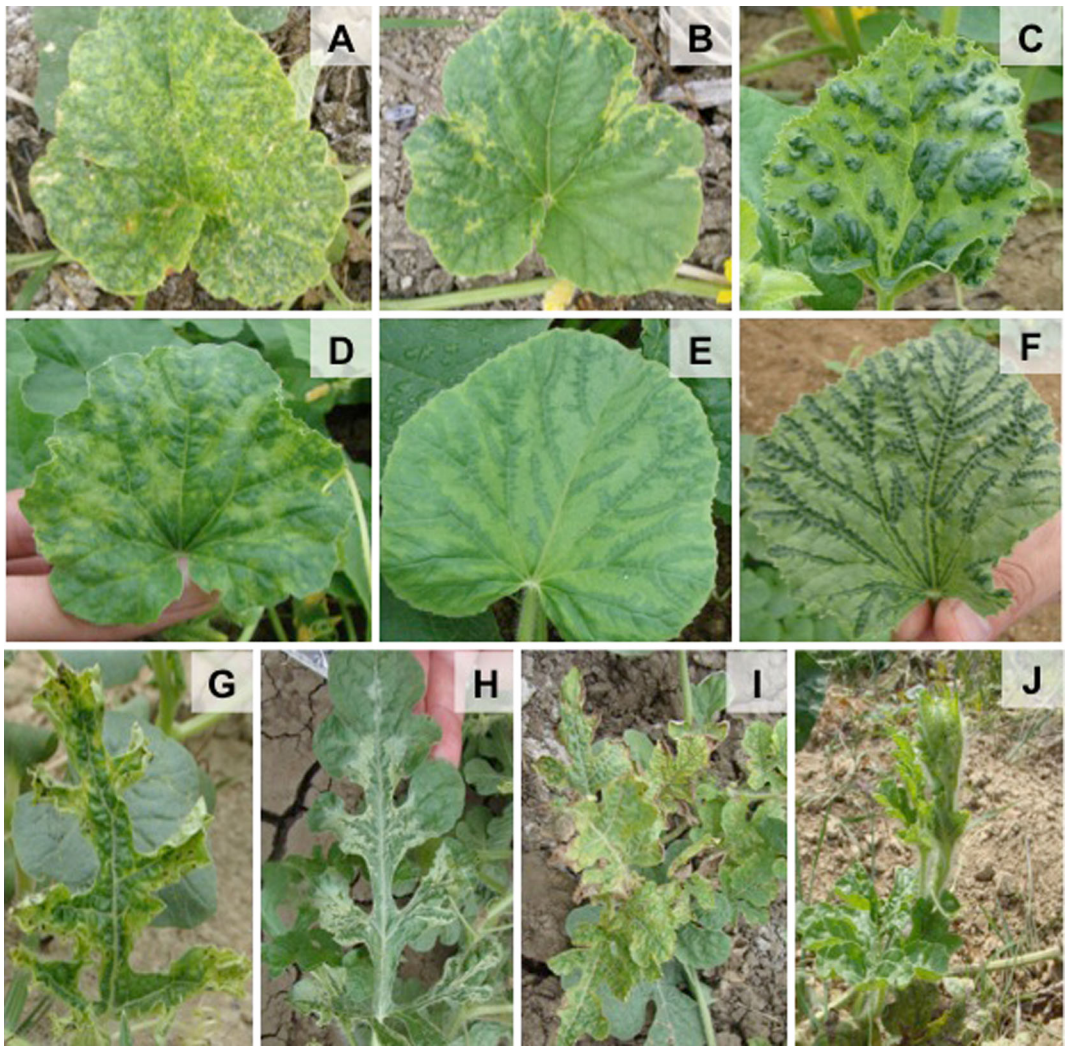


**Fig. 1** Common virus symptoms observed in melon and watermelon fields. A-F: Melon; G-J: Watermelon

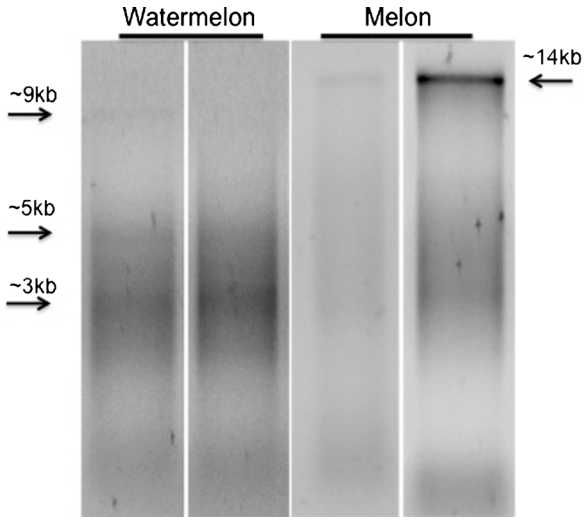


**Fig. 2** Electrophoretic profile of double-stranded RNA (dsRNA) from watermelon and melon leaves. Arrows show the location of bands at~9 kb, ~ 5 kb and~3 kb presumably the genomic RNAs of *Melon yellow spot virus* (MYSV) and~14 kb presumably the genomic RNA Cucumis melo endornavirus (CmEV)

fragments were cloned using a Strataclone PCR cloning kit (Fermentas, USA) and sequenced (Macrogen, South Korea).

Electrophoretic analysis of dsRNA revealed the presence of three bands of approximately 9 kb, 5 kb and 3 kb from watermelon; whereas an additional ~14 kb band was observed in dsRNA preparations from most melon samples (Fig. 2). In watermelon, sequencing revealed the presence of *Melon yellow spot virus* (MYSV, genus *Tospovirus*) first reported and characterized in Japan, and later found in China and Taiwan (Kato et al. 2000; Peng et al. 2011; Gu et al. 2012). In melon, Cucumis melo endornavirus (CmEV) (Coutts 2005) was found in addition to MYSV. Additional viruses were not detected. The genome size of MYSV (segmented genome composed of 8.9 kb, 4.8 kb and 3.2 kb) and CmEV (predicted genome of approximately 14 kb) suggests that the observed dsRNA electrophoretic pattern (Fig. 2) most likely shows the replicative forms of MYSV and CmEV, respectively.

The partial sequence obtained for each virus was used to design primers for the amplification of a 578 nt fragment of the MYSV S segment (MYSV-EcF: 5′-GATCAGATTGGCCAACATCACC-3′/ MYSV-EcR: 5′- GGGTGAGAGCTCTTCTAAGAG-3′) and a 413 nt fragment of CmEV (CmEVF: 5′-GGTGGAATATGGGTTGATGCTAG-3′/CmEVR: 5′-CGTCGTGATGGACATCAACTCTAC-3′). A total of 165 leaf samples (watermelon $n=72$, melon $n=93$) from symptomatic and asymptomatic plants, were collected during November of 2013 at different locations, including those where the initial sampling was done. In addition, leaf samples ($n=36$) from cucumber plants (*Cucumis sativus*, hybrid Humocaro) showing leaf yellowing and mosaic were collected from plantings near watermelon crops.

Individual samples were used for total RNA extraction as described by Halgren et al. (2007) and subjected to reverse transcription (RT) using random hexamers as recommended (Thermo Scientific, USA). Polymerase chain reaction (PCR) was done following the manufacturer's instructions (Genescript, USA). PCR parameters for the primers used in this study were as follows: 94 °C for 4 min, 40 cycles of 94 °C for 45 s, 55 °C for 30 s and 72 °C for 45 s, and a final extension step of 10 min at 72 °C. Figure 3 shows the PCR amplified products using the recommended detection primers and parameters.

MYSV was detected in 40 %, 67 % and 64 % of symptomatic watermelon, melon and cucumber samples, respectively. Sequence comparisons showed

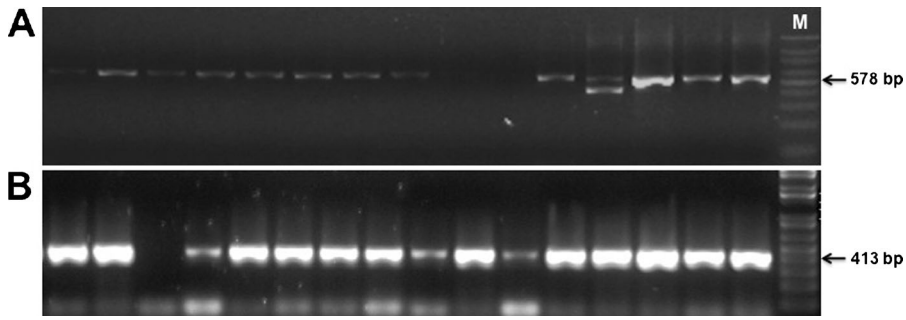


**Fig. 3** DNA electrophoresis of detection RT-PCR for *Melon yellow spot virus* (panel A) and Cucumis melo endornavirus (panel B). The size of the amplified product for each virus is indicated by the arrows

nucleotide identities of 97 % and 94 % for the RdRp and the nucleocapsid protein, respectively, between the Ecuadorean MYSV isolate (MYSV-Ec) and the Japanese isolate (Kato et al. 2000).

CmEV was detected in 95 % of melon plants tested (both symptomatic and asymptomatic). The virus was not detected in watermelon or cucumber. Alignments between CmEV-Ec and those available in the GenBank, showed 98 % identity at the nucleotide level. CmEV was not associated with any leaf symptoms. Possible effects of the endornavirus on yield and fruit quality, however, remain to be studied.

To rule out the presence of additional RNA viruses that may have been elusive to cloning and sequencing, individually collected samples were also tested for SqMV and WSMoV, by ELISA (AGDIA, USA); and CMV and potyviruses by RT-PCR using specific and potyvirus generic Nib primers, respectively (Zheng et al. 2008). All plants tested negative for SqMV and WSMoV. CMV was detected in 13 %, 73 % and 68 % of symptomatic watermelon, melon and cucumber samples, respectively. The presence of PRSV was confirmed by sequencing the Nib product and was detected in 50 %, 13 % and 14 % of symptomatic watermelon, melon and cucumber samples, respectively. MYSV, CMV and PRSV were not detected from asymptomatic plants. Generic primers designed to provisionally establish the existence of begomovirus infection failed to amplify the~575 bp fragment of the begomoviral coat protein gene (Brown et al. 2001). Table 1 summarizes the testing results obtained from this survey.

Considering that most of the viruses reported in cucurbits possess RNA genomes, the approach used in this study proved useful to identify unreported RNA viruses in Ecuador. In addition, this communication shows that MYSV and CMV are present at high percentage in tested samples and are probably associated with most of the symptoms observed in watermelon, melon and cucumber in Manabí and Santa Elena provinces of Ecuador. Unfortunately, despite a thorough picture record of symptomatic samples during this study, it was not possible to achieve a consistent pattern of symptoms potentially caused by MYSV in single or mixed infections (Table 1). Neither was it possible to determine whether symptom expression was dependent on the variety. Since most growers use different varieties and even saved seeds blended in the same field, the inconsistencies observed in symptom expression in plants infected by MYSV may be inherent to the plant genotype. Therefore, further studies under controlled conditions are needed to determine the impact of MYSV in single and mixed infections on cucurbits crops.

**Table 1** Virus occurrence in melon, watermelon and cucumber fields from Santa Elena and Manabí provinces, Ecuador. Total number of samples (n) for each crop is indicated. Virus abbreviations: SqMV: *Squash mosaic virus*; WSMoV: *Watermelon silver mottle virus*; CMV: *Cucumber mosaic virus*; PRSV: *Papaya ringspot virus*; MYSV: *Melon yellow spot virus*; CmEV: Cucumis melo endornavirus. Two or more virus abbreviations separated by '+' denote mixed infections

| Virus Detected* | Virus Occurrence (%) | | |
|---|---|---|---|
| | Melon *n= 93* | Watermelon *n= 72* | Cucumber *n= 36* |
| SqMV | 0 | 0 | 0 |
| WSMoV | 0 | 0 | 0 |
| CmEV | 5 | 0 | 0 |
| CMV | 5 | 4 | 27 |
| PRSV | 0 | 14 | 0 |
| MYSV | 0 | 4 | 18 |
| MYSV + PRSV | 0 | 27 | 5 |
| MYSV + CMV | 0 | 0 | 32 |
| MYSV + PRSV + CMV | 0 | 9 | 9 |
| CmEV + CMV | 23 | 0 | 0 |
| CmEV + MYSV | 18 | 0 | 0 |
| CmEV + PRSV | 0 | 0 | 0 |
| CmEV + CMV + MYSV | 36 | 0 | 0 |
| CmEV + CMV + PRSV | 0 | 0 | 0 |
| CmEV + PRSV + MYSV | 4 | 0 | 0 |
| CmEV + CMV + PRSV + MYSV | 9 | 0 | 0 |
| Non-infected | 0 | 42 | 9 |

* Testing method was RT-PCR for all the viruses except for SqMV and WSMoV, which were tested by ELISA (AGDIA)

This work demonstrates the occurrence of MYSV in cucurbit producing areas of Ecuador, but also highlights the importance of including MYSV in routine virus surveys in the region. A recent virus survey, conducted in cucurbit crops in Venezuela, showed that *Melon chlorotic mosaic virus* (MeCMV, genus *Begomovirus*) and the potyviruses PRSV and *Zucchini yellow mosaic virus* (ZYMV) were the most common viruses in the sampled area (Romay et al. 2014). The presence of MYSV was not assessed in that study. MYSV may be widespread in other cucurbit-producing countries in the Americas, including Venezuela. However, the lack of commercial MYSV detection kits is presumably the main reason for the lack of surveillance for the virus in this region.

To the best of our knowledge, this constitutes the first report of MYSV outside of Asia. Partial sequences of the MYSV-Ec corresponding to the RdRp, NSm and NSs have been deposited in the NCBI Genbank under accession numbers KJ196381-3, respectively. Studies on different strategies to manage MYSV by reducing the spread of thrips are underway.

**Acknowledgments** This work was partially funded by the Integrated Pest Management –Collaborative Research Support Program, IPM-CRSP (USAID sub-award number: 425,981-19A72). Additional funding was provided by the Ecuadorean Science and Technology Secretariat (SENESCYT), through the National Emblematic Program PROMETEO. The authors are thankful to all growers in Manabí and Santa Elena for granting access to fields for sample collection.

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