A New *Tospovirus* sp. in Cucurbit Crops in Mexico

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ABSTRACT

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During the 2007 growing season, melon (*Cucumis melo*) samples from the state of Guerrero in Mexico showing mosaic and other virus-like symptoms were collected for analysis. Electron microscopic examination of negatively stained leaf-dip extracts revealed the presence of abundant virus-like particles with features characteristic of the family *Bunyaviridae*. No other viral particles were observed in these preparations. However, enzyme-linked immunosorbent assays (ELISAs) specific for the most common *Tospovirus* spp. gave negative results. Antibodies raised against purified nucleocapsids reacted specifically with the infected leaf extracts in Western blots and double-antibody sandwich ELISA. The viral RNA was used as a template for a cDNA library, and nucleotide sequence analysis identified cloned cDNAs representing sequences corresponding to the three *Tospovirus* genome segments. Sequence comparisons showed that the new virus had the highest similarity to Chrysanthemum stem necrosis virus (CSNV). Phylogenetic analysis of two genome regions confirmed that this virus, provisionally named Melon severe mosaic virus (MeSMV), is a previously undescribed *Tospovirus* sp. belonging to the "new world" clade of *Tospovirus* spp. An initial survey of various cucurbit crops in various states of Mexico confirmed the widespread occurrence of this virus.

The genus *Tospovirus* (*Bunyaviridae*) (9,18) includes some of the most important plant viruses causing severe diseases in agricultural, ornamental, and horticultural crops (16,26,34). The type member of the genus is *Tomato spotted wilt virus* (TSWV; 20). *Tospovirus* spp. possess a tripartite single-stranded RNA genome with a negative or ambisense expression strategy (34). Genomic RNAs are encapsidated by nucleocapsid proteins (N) and enveloped by a lipoprotein membrane (34).

The three genome segments are referred to as small (S), medium (M), and large (L). The S segment encodes a nonstructural protein (NSs) in the viral sense (2,11,31) and the N protein in the viral complementary sense (11). The M segment encodes in the viral sense a nonstructural protein (NSm) (28,29) and, in the viral complementary sense, the precursor of the two-membrane glycoproteins (27,38,39). The L segment encodes the RNA-dependent RNA polymerase protein in the viral complementary sense (11).

Cucurbit-infecting *Tospovirus* spp. have been identified in different countries since the 1980s, particularly in South America and Asia. Two cucurbit-infecting *Tospovirus*

spp. are included in the list of the species accepted by the International Committee on Taxonomy of Viruses (ICTV): *Watermelon silver mottle virus* (WSMoV), originally reported on *Citrullus lanatus* in Okinawa, Japan in 1982 and in 1992 in Taiwan (40); and *Zucchini lethal chlorosis virus* (ZLCV), reported on *Cucurbita pepo* in Brazil in 1999 (1). In addition, two tentative species from cucurbits have been proposed: *Watermelon bud necrosis virus* (WBNV), reported on cucurbits in India (13); and, more recently, *Melon yellow spot virus* (MYSV) on netted melon in Japan (14).

In the state of Guerrero, Mexico, several honeydew melon (*Cucumis melo*) plants exhibiting mosaic, leaf blistering, leaf deformation, and fruit splitting were observed. Electron-microscopic analysis of leaf-dip extracts showed only particles with the morphological features of viruses of the *Tospovirus* genus. Biological, serological, and molecular properties and phylogenetic analysis showed that this *Tospovirus* sp. is a new and distinct species in the genus *Tospovirus* and is tentatively named Melon severe mosaic virus (MeSMV). Surveys showed its widespread occurrence in cucurbit crops in Mexico.

MATERIALS AND METHODS

Virus isolation and maintenance. The virus was isolated from symptomatic honeydew melon (*C. melo*) plants during the 2007 melon-growing season in the state of Guerrero, Mexico. Symptomatic plants were first checked for TSWV presence by specific double-antibody sandwich en-

zyme-linked immunosorbent assay (DAS-ELISA) using a commercial kit from Agdia (Elkhart, IN) and our own DAS-ELISA kit (24). The virus was mechanically transferred onto *C. melo* and *Nicotiana benthamiana* plants, and leaves were stored in liquid nitrogen to be used as the source of successive mechanical inoculations. To avoid the possibility of generating deletions or mutations in the virus genome, mechanical inoculations were limited to not more than three to four serial passages. The presence of *Tospovirus* sp.-like virions in infected plants was confirmed by electron-microscopic observation (Philips CM10, Eindhoven, the Netherlands) of crude sap negatively stained in 0.5% aqueous uranyl acetate.

Host range. Leaves of symptomatic *C. melo* and *N. benthamiana* plants showing strong mosaic symptoms 2 weeks post inoculation were homogenized with 50 mM phosphate buffer, pH 7, containing 1 mM Na-EDTA, 5 mM Na-DIECA, and 5 mM Na-thioglycolate (extraction buffer) and mechanically inoculated onto leaves of a series of herbaceous plants to determine the partial host range (Table 1) as previously described (35). Plants were grown in an insect-proof glasshouse at a variable temperature between 20 and 25°C, and three individual plants for each species were inoculated when the first true leaves were fully expanded; symptoms were monitored after 1 and 4 weeks post inoculation. DAS-ELISA (as described below) was used to confirm the presence of MeSMV in both symptomatic and asymptomatic inoculated plants.

Nucleocapsid purification and antiserum production. Viral nucleocapsids were purified from 100 g of MeSMV-infected *N. benthamiana* plants following the method used for other *Tospovirus* spp. (23,24). The partially purified preparation was then layered on a 10- to 50-% sucrose gradient and centrifuged at 270,000 × g for 2 h. A band corresponding to the nucleocapsids was collected, and purity was assessed by electron microscopy and by Coomassie staining of the samples separated in 10% tris-tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described (35).

A polyclonal antiserum was obtained from a 'New Zealand' rabbit injected intramuscularly with the nucleocapsid suspension. Purified nucleocapsid (1 mg) emulsified with Freund's complete adjuvant (1:1, vol/vol) was used for the first

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injection. Booster injections were given after 2, 4, and 6 weeks with incomplete Freund's adjuvant. Blood was collected three times, every 15 days, beginning 2 weeks from the second injection. Serum was separated after overnight incubation at 4°C and cross-absorbed with healthy plant extract (*N. benthamiana*). Immunoglobulin G (IgG) was purified through affinity chromatography on protein G columns (Pharmacia, Skokie, IL) according to the manufacturer's instructions.

ELISA and Western blot analysis. The specificity of purified MeSMV-IgG was assessed by antigen-coated plate (ACP) ELISA using dilutions of infected and healthy plants (35). An aliquot of the IgG was conjugated with alkaline phosphatase using the glutaraldehyde two-step method and DAS-ELISA was performed as previously detailed (23).

For the Western blot analysis, purified nucleocapsid preparations or total leaf protein extracts were separated by 10% SDS-PAGE and transferred to 0.45-µm nitrocellulose parablots NCP membranes (Macherey-Nagel, Duren, Germany) as described (36). Purified IgGs diluted 1:1000 in TBS-Tween + 7% dried skimmed milk were used as primary antibody, and anti-rabbit IgG conjugated with horseradish peroxidase (Sigma-Aldrich, St. Louis; dilution 1:5000 in TBS-Tween + 7% dried skimmed milk) was used as the secondary antibody. Super Signal West Pico Chemiluminescent substrate (Pierce, Rockford, IL) was used following the manufacturer's instructions to detect the presence of bound secondary antibodies.

RNA extraction, cDNA cloning, and sequence analyses. Total RNA was extracted from purified nucleocapsids using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The Universal RiboClone Synthesis System (Promega Corp., Madison, WI) was used to obtain complementary DNA (cDNA) following the protocol provided by the manufacturer for first- and second-strand synthesis. A step including adaptor ligation and further polymerase chain reaction (PCR) amplification of the cDNA was performed as previously described (37). Selected PCR fragments were purified (GeneClean; qBiogene, Irvine, CA) and cloned in pGEM-T easy vector (Promega Corp.), and white colonies were screened by PCR with M13 forward and reverse primers. Positive colonies were grown in 4 ml of Luria-Bertani with ampicillin at 0.05 mg/ml, and plasmids were purified with the QIAprep spin Miniprep kit (Qiagen, Hilden, Germany). Plasmid inserts were sequenced using the dideoxy chain terminator method (25).

In order to obtain the full-length S segment sequence, reverse transcription on

purified viral RNAs was performed using the ThermoScript reverse-transcription (RT)-PCR System (Invitrogen) using random hexamers to prime cDNA synthesis. PCR was then performed using primers designed from initial S-segment genomic fragment sequences (Table 2). Selected PCR products were purified (Qiagen), cloned in pGEM-T Easy, and sequenced. The S segment 5' and 3' ends were cloned using the oligonucleotide J13 generic for *Tospovirus* ends as previously described (6) and internal specific primers (Table 2).

Clustal X version 1.81 was used for multiple sequence alignments (32). Statistical significance was calculated by performing 100 replications of bootstrap resampling of the original alignment using SEQBOOT. Phylogenetic trees were constructed with the PROTML algorithm in the Phylip version 3.6 (10) using the Dayhoff substitution matrix, one randomized sequence—input order, the global rearrangement option, and a mixed heterogeneity among sites variation model (one invariable plus six Gamma rates). TREE-PUZZLE 5.0 (30) was used to estimate the relative rate for each category. Trees were

Table 2. List of Melon severe mosaic virus (MeSMV) primers used to complete the small (S) RNA sequence and for MeSMV detection by reverse-transcription polymerase chain reaction (primers MeSMV-2490-F and MeSMV-2900-R)

Primer name	Sequence
MeSMV-2490-F	5' tgctgaggatttgcacatcatt 3'
MeSMV-2900-R	5' gggatcacatcaaaaaatacaaac 3'
MeSMV-2510-R	5' tggataatgatcaactgg 3'
MeSMV-1380-F	5' gtcttcagcctccagagc 3'
MeSMV-1020-R	5' cctaaacagggttaagttt 3'

Table 1. Partial host range analysis of Melon severe mosaic virus^a

Family	Species	Symptoms		ELISA results	
		Inoculated leaves	Systemic	Local	Systemic
Solanaceae	<i>Datura stramonium</i>	cII	0	+	—
	<i>Capsicum annuum</i> cv. Q.A.	cII	0	+	—
	<i>C. chinense</i> 152225	0	0	—	—
	<i>Solanum lycopersicum</i> cv. York	0	0	—	—
	<i>S. lycopersicum</i> cv. Marmande	0	0	—	—
	<i>Nicotiana clevelandii</i>	nII	Deformation/mosaic	+	+
	<i>N. megalosiphon</i>	nII	Necrosis/deformation	—	+
	<i>N. rustica</i>	0	0	+	—
	<i>N. tabacum</i> cv. White Burley	cII/nII	0	+	—
	<i>N. glutinosa</i>	nII	0	—	—
	<i>N. benthamiana</i>	cII/nII	Mosaic/deformation	+	+
	<i>Emilia sonchifolia</i>	0	Chlorotic spot	+	+
	<i>Petunia × hybrida</i>	nII	0	—	—
	<i>Cucurbita pepo</i> cv. Genovese	0	0	—	—
Cucurbitaceae	<i>Cucumis melo</i>	cII	Mosaic/deformation	+	+
	<i>Cucumis sativus</i>	0	0	—	—
	<i>Vinca rosea</i>	0	0	—	—
Apocynaceae	<i>Ocimum basilicum</i>	0	0	—	—
Leguminosae	<i>Phaseolus vulgaris</i> cv. Saxa	0	0	—	—
Chenopodiaceae	<i>Chenopodium quinoa</i>	0	0	—	—
	<i>Chenopodium amaranticolor</i>	0	0	—	—
Amaranthaceae	<i>Beta vulgaris</i>	cII/nII	0	+	—
	<i>Gomphrena globosa</i>	0	0	+	—

^a Three individual plants for each species tested were mechanically inoculated when their first true leaves were fully expanded using leaf sap from symptomatic *Nicotiana benthamiana* and *Cucumis melo* leaves collected 2 weeks post inoculation. Host range results are based on visual evaluation and confirmed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) results (visual evaluation was the same for each of the three plants and a mixed sample for each species was evaluated by DAS-ELISA). CII = chlorotic local lesion; nII = necrotic local lesion; 0 = no symptom; + and — symbols correspond to infected/healthy ratio (I/H) of absorbance values above or below 3 in DAS-ELISA.

generated with TreeView (19). Amino acid sequences of the other *Tospovirus* spp. used for the analysis were taken from GenBank, EMB, DDBJ, and PDB databases. RNA secondary structures were predicted using the Mfold web server (41).

Survey of Mexican cucurbits crops. In February 2008, a survey of cucurbit crops across Mexico was carried out in four different regions, ranging from Sinaloa in the northwest to the state of Chiapas in the south. In all, 147 plants showing possible virus-caused symptoms (including *C. melo*, *Cucurbita pepo*, *Cucumis sativus*, and *Citrullus lanatus*) were collected and analyzed throughout the growing season (February to May) for the presence of the new *Tospovirus* sp. by using MeSMV-specific lateral flow (LF) devices. Commercial LF devices were prepared by Agdia from our nucleocapsid antibodies and used for the field survey. Symptomatic plant development stage ranged from

flowering to ripe fruit ready for harvest. For confirmation of the serological analysis, RT-PCR was carried out on 30 RNA samples extracted from leaves as described above using the oligonucleotides MeSMV-2490F and MeSMV-2900R (Table 2), with 30 cycles of PCR using a denaturing step of 30 s at 94°C, an annealing step at 55°C for 30 s, and a step for polymerase activity at 72°C for 20 s. Ten samples (including positives and negatives by LF) were also analyzed by DAS-ELISA for further validation.

RESULTS

Virion morphology, symptoms, and partial host range. Enveloped pleomorphic particles with features typical for members of the *Tospovirus* genus were observed by electron microscopy of leaf extracts of symptomatic melon plants. Similar particles were seen from plants showing a variety of symptoms, including

an early onset of mosaic and leaf blistering symptoms on honeydew melon leaves (Fig. 1A); advanced blistering, mosaic, and leaf deformation on honeydew melon (Fig. 1B); necrotic lesions on mature honeydew melon (Fig. 1C); and immature honeydew fruit exhibiting necrotic fruit splitting (Fig. 1D). No other virus-like particles were observed or detected by other methods, including DAS-ELISA, for the most common cucumo- and potyviruses present in the region, such as *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic* (ZYMV), and *Cucumber mosaic virus* (CMV). When leaves were used for mechanical inoculation of *N. benthamiana* and *Cucumis melo* plants, symptoms were clear on inoculated plants by 7 days post inoculation (dpi). In fact, severe symptoms similar to those seen on the original diseased plants, such as mosaic, leaf deformation, and growth arrest, were observed.

Fig. 1. Symptoms of melon plants infected with Melon severe mosaic virus. Photographs were taken in February 2007 and February 2008 in a field in Guerrero State, Mexico. **A**, Early onset of mosaic and leaf blistering symptoms on honeydew melon leaves; **B**, advanced blistering, mosaic, and leaf deformation on honeydew melon; **C**, necrotic lesions on mature honeydew melon; and **D**, and immature honeydew fruit exhibiting necrotic fruit splitting.

MeSMV was able to systemically infect plants of *N. benthamiana*, *N. megalosiphon*, *Datura stramonium*, and *N. clelandii*, and caused deformation, mosaic, or necrosis; the virus infection caused also chlorotic spots on *Emilia sonchifolia* plants. DAS-ELISA confirmed the presence of MeSMV in inoculated and uninoculated leaves (Table 1). Interestingly, MeSMV did not infect the other

two *Cucurbitaceae* spp. present in the host range study: *C. sativus* and *Cucurbita pepo* cv. Genovese.

Virus purification and serology. Typical *Tospovirus* sp.-like nucleocapsids were observed by electron microscopy in negatively stained purified MeSMV nucleocapsid preparations. The molecular weight of the N protein was estimated to be about 30 kDa by SDS-PAGE (Fig. 2).

Fig. 2. Coomassie stained 10% polyacrylamide gel showing purified nucleocapsid proteins of Melon severe mosaic virus (MeSMV), *Impatiens necrotic spot virus* (INSV), *Polygonum ringspot virus* (PolRSV), and *Tomato spotted wilt virus* (TSWV). MWM, molecular weight markers. Numbers at left indicate positions for proteins of the respective size ($\times 10^{-3}$).

In DAS-ELISA, the MeSMV antibody did not cross-react with other *Tospovirus* antigens tested, whereas some cross-reactivity was shown for a number of *Tospovirus* antigens (including a very low value for MeSMV) when anti-CSNV and anti-TCSV antibodies were used (Fig. 3). Western blot analysis using anti-MeSMV antibodies on total soluble proteins extracted from infected leaves confirmed the serological reactions seen in DAS-ELISA. MeSMV antibodies showed a strong reaction with its corresponding infected plant but also a positive reaction with TSWV-infected plants (Fig. 4). However, TSWV antibodies (A353) did not show a similar cross-reaction with MeSMV in Western blot (Fig. 4).

Sequence analysis and virus phylogenetic and taxonomic placement. The MeSMV complete nucleotide sequence of the S RNA was obtained from a number of overlapping clones (National Center for Biotechnology Information [NCBI] accession no. EU275149). The S RNA was 3,283 nucleotides (nt) and contained two open reading frames (ORF). The first ORF, from nucleotide 81 to 1,448, encoded the NSs protein (455 amino acids [aa]) with an estimated molecular weight of 50.9 kDa. The second ORF, from nucleotide 2,336 to 3,124, encoded the N protein (262 aa) with an estimated molecular weight of 29.5 kDa, which was similar to the N protein molecular weight estimate based on electrophoretic mobility of the purified nucleocapsids. The noncoding intergenic

Fig. 3. Serological reactions in double-antibody sandwich enzyme-linked immunosorbent assay among six *Tospovirus* spp. using antisera made against the nucleocapsids of each virus (X axis) and, as antigens, *Nicotiana benthamiana* plants infected with each of the homologous viruses detailed in the legend. Numbers in the Y axis indicate absorbance values at 405 nm. Error bars represent standard deviation calculated from four replicates for each sample. Viruses: *Tomato spotted wilt virus* (TSWV) BAA03025, *Tomato chlorotic spot virus* (TCSV) AAG23654, *Impatiens necrotic spot virus* (INSV) AAA47974, *Chrysanthemum stem necrosis virus* (CSNV) AAF04197, *Melon severe mosaic virus* (MeSMV), and *Iris yellow spot virus* (IYSV) AAF75556.

region (IR) was 888 nt, rich in A and U residues (74%), and predicted to form the typical hairpin secondary structure (*not shown*). The sequences of a 1-kb fragment of the M RNA and a 1.5-kb fragment of the L RNA were also obtained and deposited in GenBank (NCBI accession nos. FJ157984 and FJ157985, respectively).

To clarify the taxonomic position of MeSMV, the N protein and a part of the glycoprotein (G) precursor protein (from amino acid position 430 to 917 when aligned to TSWV G precursor protein, therefore covering part of the Gc region) were compared with corresponding regions of other known *Tospovirus* spp. For the N protein, the highest similarity was with CSNV (67%; Table 3). For partial G protein, the highest similarity was with TSWV (72%; Table 4). A phylogenetic tree based on *Tospovirus* N proteins (Fig. 5A) showed the presence of four main clusters: one with viruses distributed mainly in North and South America, assuming that TSWV and *Impatiens necrotic spot virus* (INSV) are likely of American origin; a second cluster of the tospoviral species from Asia; a third cluster containing Iris yellow spot virus (IYSV), Polygonum ring spot virus (PolRSV), and Tomato yellow ring virus (TYRV), three *Tospovirus* spp. likely of Middle-Eastern origin (12); and a fourth cluster composed of Peanut yellow spot virus (PYSV) and Peanut chlorotic fan-spot virus (PCFV), two viruses with possible origin in south-eastern Asia. MeSMV is in the clade of American species but in a separate branch. The consensus tree relative to G protein alignments (Fig. 5B) showed that MeSMV clusters with “new world” *Tospovirus* spp.

Survey of Mexican cucurbits crops. Following the initial identification of MeSMV as a new *Tospovirus* sp., a survey to assess its incidence and distribution in several melon-growing areas of Mexico was undertaken. Of the 147 symptomatic plants that were tested, MeSMV was found in melon, zucchini, cucumber, and watermelon plants (Table 5). The results from LF were confirmed when validated by both DAS-ELISA (carried out on 10 samples) and RT-PCR (carried out on 30 samples).

Given the surprising results for the field-infected cucumber sample, we checked whether significant molecular differences were present in the cucumber isolate when compared with the melon isolate we had cloned and sequenced; the RT-PCR product obtained from the same sample was sequenced directly, confirming 99% identity with the MeSMV sequence deposited in GenBank (*not shown*). In summary, the majority of the 147 plants (66%) from various states in Mexico were MeSMV positive by LF, indicating its relatively wide geographic distribution and presence in different cucurbit crops (Table 5).

DISCUSSION

Cantaloupe is one of the most economically important cucurbit crops in Mexico. PRSV, WMV, ZYMV, and CMV

are the major viruses reported in Mexican cucurbit crops (8). *Squash leaf curl virus* is also prevalent in some parts of Mexico (3,5,22) and, recently, *Cucurbit yellow*

Fig. 4. Western blot analysis of leaf extracts from *Nicotiana benthamiana* plants infected with *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), and Melon severe mosaic virus (MeSMV) using antibodies against the nucleocapsid of TSWV and MeSMV. Lower panel is a Coomassie stain of the gel to show the amount of total protein loaded.

stunting disorder virus has been reported (4).

In recent years, a new disease has been spreading in cucurbits crops, especially on melon and watermelon, sometimes reducing production by as much as 20 to 30%. Our study showed that a new *Tospovirus* sp. is associated with this new disease. Specificity of the host range and serological and N-protein relationship indicate that MeSMV is a new *Tospovirus* sp. It was somewhat surprising to confirm MeSMV infection of zucchini and cucumber in the open fields, because plants of these species did not get infected in our host range ex-

periments. This could be due to several reasons, including different cultivars, mechanical versus thrips transmission, or biological diversity of the virus isolates from different hosts, even though no evidence of such occurrence was shown for other *Tospovirus* spp. (33). Nevertheless, MeSMV has a broad host range among cucurbits and could represent a new virus problem in the Americas.

The N protein amino acid sequence is an important criterion to classify *Tospovirus* spp. (7,9,15,21). Less than 80% similarity is considered the limit to separate different species (15). Analysis of the S RNA ge-

nomic segment showed that maximum similarity between the MeSMV N protein amino acid sequence and those of other *Tospovirus* spp. is 67% with CSNV, well below the threshold and, thus, supporting the idea that MeSMV is a distinct *Tospovirus* sp.

Like our host range and sequence analyses, serological analysis for *Tospovirus* N proteins showed that MeSMV is a distinct *Tospovirus* sp. Our Western blot analysis showed that MeSMV antibodies cross-reacted with TSWV N proteins but only when tested under these denaturing conditions. This warns against using this tech-

Table 3. Similarity based on the alignment of the nucleocapsid protein of 22 *Tospovirus* spp.^a

Virus	GloRSV	CaCV	TomNecrV	GBNV	PBNV	WBNV	WSMoV	CCSV	MYSV	PhySMV	PolRSV	TYRV	IYSV	CSNV	ZLCV	GRSV	TCSV	TSWV	MeSMV	INSV	PCFV	PYSV
GloRSV	1.000	1.000	0.974	0.888	0.887	0.841	0.886	0.707	0.629	0.635	0.488	0.528	0.509	0.270	0.277	0.283	0.282	0.294	0.288	0.313	0.274	0.238
CaCV	...	1.000	0.974	0.888	0.887	0.841	0.886	0.709	0.633	0.638	0.493	0.533	0.514	0.277	0.284	0.290	0.288	0.301	0.286	0.319	0.271	0.235
TomNecrV	...	...	1.000	0.880	0.880	0.845	0.890	0.684	0.631	0.637	0.491	0.531	0.516	0.272	0.280	0.286	0.284	0.302	0.286	0.306	0.282	0.243
GBNV	...	...	...	1.000	0.996	0.883	0.907	0.691	0.608	0.608	0.469	0.489	0.452	0.272	0.295	0.281	0.270	0.272	0.309	0.306	0.256	0.227
PBNV	...	...	...	...	1.000	0.886	0.906	0.691	0.613	0.613	0.473	0.493	0.457	0.275	0.298	0.284	0.272	0.275	0.312	0.299	0.259	0.227
WBNV	...	...	...	...	...	1.000	0.886	0.686	0.575	0.575	0.457	0.468	0.436	0.277	0.290	0.276	0.250	0.262	0.304	0.296	0.249	0.213
WSMoV	...	...	...	...	...	...	1.000	0.703	0.610	0.615	0.475	0.500	0.459	0.273	0.305	0.296	0.256	0.259	0.311	0.306	0.247	0.246
CCSV	...	...	...	...	...	...	...	1.000	0.630	0.630	0.486	0.530	0.484	0.260	0.282	0.262	0.222	0.254	0.278	0.245	0.200	0.192
MYSV	...	...	...	...	...	...	...	...	1.000	0.992	0.524	0.527	0.536	0.316	0.309	0.300	0.288	0.304	0.299	0.299	0.201	0.197
PhySMV	...	...	...	...	...	...	...	...	...	1.000	0.529	0.532	0.541	0.319	0.312	0.302	0.291	0.307	0.302	0.301	0.201	0.199
PolRSV	...	...	...	...	...	...	...	...	...	...	1.000	0.825	0.709	0.377	0.385	0.382	0.371	0.348	0.343	0.311	0.240	0.261
TYRV	...	...	...	...	...	...	...	...	...	...	...	1.000	0.722	0.371	0.410	0.412	0.390	0.367	0.356	0.300	0.223	0.244
IYSV	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.332	0.330	0.350	0.353	0.405	0.338	0.308	0.190	0.236
CSNV	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.814	0.776	0.781	0.782	0.670	0.580	0.254	0.268
ZLCV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.775	0.744	0.708	0.642	0.571	0.235	0.230
GRSV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.863	0.761	0.636	0.587	0.253	0.257
TCSV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.785	0.638	0.564	0.235	0.260
TSWV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.570	0.598	0.230	0.239
MeSMV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.569	0.240	0.281
INSV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.232	0.244
PCFV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.708
PYSV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000

^a Virus acronyms: Gloxinia ringspot virus (GloRSV) AAQ83791, Capsicum chlorosis virus (CaCV) ABC86907, Tomato necrosis virus (TomNecrV) AAT68025, Groundnut bud necrosis virus (GBNV) AAR24021, Peanut bud necrosis virus (PBNV) AAM76063, Watermelon bud necrosis virus (WBNV) ABD39046, Watermelon silver mottle virus (WSMoV) AAW64930, Calla lily chlorotic spot (CCSV) AAW58115, Melon yellow severe virus (MYSV) BAB79455, Physalis severe mosaic virus (PhySMV) AAD34201, Polygonum ringspot virus (PolRSV) EF445397, Tomato yellow ring virus (TYRV) ABF59486, Iris yellow spot virus (IYSV) AAF75556, Chrysanthemum stem necrosis virus (CSNV) AAF04197, Zucchini lethal chlorosis virus (ZLCV) AAF04198, Groundnut ring spot virus (GRSV) AAF25255, Tomato chlorotic spot virus (TCSV) AAG23654, Tomato spotted wilt virus (TSWV) BAA03025, Melon severe mosaic virus (MeSMV), *Impatiens necrotic spot virus* (INSV) AAA47974, Peanut chlorotic fanspot virus (PCFV) AAC99405, and Peanut yellow spot virus (PYSV) AAB94022. Similarity values were obtained by the software PROTDIST as detailed in the material and methods section. In bold are the values relative to MeSMV nucleocapsid alignment with the other *Tospovirus* spp.

Table 4. Similarities between a fragment of the glyco- protein of 15 *Tospovirus* spp.^a

Virus	CSNV	TSWV	GRSV	TCSV	ZLCV	INSV	MeSMV	GTV	CaCV	GBNV	WSMoV	TZSV	MYSV	IYSV	PolRSV
CSNV	1.000	0.907	0.862	0.865	0.874	0.708	0.694	0.393	0.384	0.394	0.385	0.388	0.405	0.403	0.420
TSWV	...	1.000	0.867	0.883	0.870	0.719	0.720	0.398	0.395	0.401	0.414	0.402	0.419	0.412	0.424
GRSV	...	...	1.000	0.975	0.831	0.706	0.715	0.406	0.389	0.399	0.414	0.398	0.403	0.411	0.403
TCSV	...	...	...	1.000	0.844	0.712	0.710	0.407	0.390	0.394	0.410	0.399	0.403	0.411	0.403
ZLCV	...	...	...	...	1.000	0.692	0.674	0.393	0.388	0.394	0.400	0.394	0.419	0.412	0.429
INSV	...	...	...	...	...	1.000	0.670	0.399	0.398	0.397	0.395	0.394	0.406	0.384	0.411
MeSMV	...	...	...	...	...	...	1.000	0.402	0.399	0.399	0.402	0.395	0.397	0.417	0.411
GTV	...	...	...	...	...	...	...	1.000	0.929	0.918	0.903	0.770	0.726	0.741	0.746
CaCV	...	...	...	...	...	...	...	...	1.000	0.905	0.881	0.764	0.745	0.738	0.727
GBNV	...	...	...	...	...	...	...	...	...	1.000	0.930	0.791	0.738	0.759	0.733
WSMoV	...	...	...	...	...	...	...	...	...	...	1.000	0.791	0.732	0.770	0.735
TZSV	...	...	...	...	...	...	...	...	...	...	...	1.000	0.749	0.706	0.724
MYSV	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.671	0.675
IYSV	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000	0.861
PolRSV	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1.000

^a Virus acronyms: Chrysanthemum stem necrosis virus (CSNV) AAF04197, Tomato spotted wilt virus (TSWV) BAA03025, Groundnut ring spot virus (GRSV) AAF25255, Tomato chlorotic spot virus (TCSV) AAG23654, Zucchini lethal chlorosis virus (ZLCV) AAF04198, *Impatiens necrotic spot virus* (INSV) AAA47974, Melon severe mosaic virus (MeSMV), Gloxinia tospovirus (GTV) (AAC15466.1), Capsicum chlorosis virus (CaCV) ABC86907, Groundnut bud necrosis virus (GBNV) AAR24021, Watermelon silver mottle virus (WSMoV) AAW64930, Tomato zonate spot virus (TZSV), Melon yellow severe virus (MYSV) BAB79455, Iris yellow spot virus (IYSV) AAF75556, and Polygonum ringspot virus (PolRSV) EF445397. Similarity values were obtained by the software PROTDIST as detailed in the material and methods section. In bold are the values relative to MeSMV nucleocapsid alignment with the other *Tospovirus* spp.

nique for open-field diagnostic purposes. By contrast, the specificity of our MeSMV antibodies in DAS-ELISA well suits the requirements for a sensitive and specific diagnostic kit to process a large number of field samples.

The vector for MeSMV is not yet known, although western flower thrips (*Frankliniella occidentalis*), a known *Tospovirus* vector species, was present on MeSMV-infected plants. CSNV is transmitted by *F. occidentalis* and *F. schultzei* (1), whereas ZLCV (another cucurbit-infecting *Tospovirus* sp. from the Americas) is transmitted only by *F. zucchini* (17).

It is unknown exactly how long MeSMV has been present in Mexico because symp-

toms are not always specific, and the presence of the most common cucurbit *Potyvirus* spp. (e.g., ZYMV) could have masked the presence of MeSMV. The widespread

presence of MeSMV on different cucurbit crops and in different states of Mexico also suggests that MeSMV has already been established and, given the contiguous na-

Table 5. Survey of Mexican cucurbit crops in February 2008

Locations in Mexico	Number of MeSMV-positive samples over the number tested ^a			
	Watermelon	Melon	Cucumber	Zucchini
Puebla	...	...	...	28/35
Guerrero, Michoacan de Ocampo	...	43/55	...	...
Campeche, Chiapas and Jalisco	15/30	...	...	...
Sinaloa	...	...	1/2	...
Mexico	...	...	...	13/25

^a Samples were analyzed by later flow devices in the field, and a subset was confirmed to be infected by Melon severe mosaic virus (MeSMV) by reverse-transcription polymerase chain reaction and double-antibody sandwich enzyme-linked immunosorbent assay.

Fig. 5. Phylogenetic analysis of Melon severe mosaic virus (MeSMV) proteins. **A**, Consensus phylogenetic tree obtained from alignments of the nucleocapsid (N) protein sequences of 21 *Tospovirus* spp., including accepted and tentative species: Capsicum chlorosis virus (CaCV) ABC86907, Calla lily chlorotic spot (CCSV) AAW58115, Chrysanthemum stem necrosis virus (CSNV) AAF04197, Groundnut bud necrosis virus (GBNV) AAR24021, Gloxinia ringspot virus (GloxRSV) AAQ83791, Groundnut ring spot virus (GRSV) AAF25255, Iris yellow spot virus (IYSV) AAF75556, Melon yellow severe virus (MYSV) BAB79455, Peanut bud necrosis virus (PBNV) AAM76063, Peanut chlorotic fanspot virus (PCFV) AAC99405, Polygonum ringspot virus (PoIRSV) EF445397, Physalis severe mosaic virus (PhySMV) AAD34201, Peanut yellow spot virus (PYSV) AAB94022, Tomato chlorotic spot virus (TCSV) AAG23654, Tomato necrosis virus (TomNecrV) AAT68025, Tomato spotted wilt virus (TSWV) BAA03025, Tomato yellow ring virus (TYRV) ABF59486, Watermelon bud necrosis virus (WBNV) ABD39046, Watermelon silver mottle virus (WSMoV) AAW64930, Zucchini lethal chlorosis virus (ZLCV) AAF04198, *Impatiens necrotic spot virus* (INSV) AAA47974, and *Bunyamwera virus* NP_047213. PBNV is now considered a strain of GBNV (10). **B**, Consensus phylogenetic tree obtained from alignments of G protein sequence fragments of 15 *Tospovirus* spp. or isolates. Branches at nodes with a bootstrap value <50 have been collapsed to polytomy. Viruses: *La crosse virus* (AAB62803.1), GBNV (NP_619703.1), Gloxinia tospovirus (GTV) (AAC15466.1), GRSV (AAU10600.2), INSV (ABD93457.1), MYSV (YP_717935.1), PoIRSV (ABX65310.1), TCSV (AAU10599.2), Tomato zonate spot virus (TZSV) (YP_001740046.1), TSWV (AAV28056.1), WSMoV (NP_620767.1), ZLCV (BAF62147.1), IYSV (AAL62023.1), CaCV (YP_717926.1), and CSNV (BAF62146.1).

ture of cucurbit cultivation between Mexico and the southern states of the United States, a close inspection of cucurbit crops in the southern United States to determine the presence of MeSMV should be undertaken.

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