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Cucumber mosaic virus infecting 'Cavendish' banana in Argentina

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Abstract

Banana plants (*Musa* sp.) showing severe mosaic symptoms in leaves were observed in Laguna Naineck, Formosa, Argentina. Electron microscopy observations of leaf dip preparations revealed isometric particles ca. 30 nm in diameter, which were identified as cucumber mosaic virus (CMV) by enzyme-linked immunosorbent assay (ELISA) test and reverse transcription-polymerase chain reaction (RT-PCR). CMV was mechanically transmitted to healthy *Nicotiana glutinosa* plants. Sequence analysis of coat protein gene showed highest nucleotides identity with other CMV isolates from United States of America, Serbia, Russia, Democratic Republic of Congo, Iran and Brazil, ranging between 96.9–98.3%. Phylogenetic analysis indicated that the virus isolate named Laguna Naineck (MH716245) belongs to the CMV group IA. To our knowledge, this is the first report of CMV in banana in Argentina. Moreover, constitutes the first Argentine CMV isolate available in the international nucleotide sequence databases.

Keywords Cucumber mosaic virus · Banana crops · Sequence analysis

In Argentina, the banana production area, located in the northern provinces, covers approximately 5400 ha. The banana is the most consumed fruit in Argentina and approximately 20% of the volume consumed corresponds to national production (Tapia and Fagiani 2015; Molina 2016). Viruses are assuming greater importance in *Musa* cultivation due to the current worldwide movement of its germplasm. Among viruses reported to infect banana, banana streak virus (BSV), banana mild mosaic virus (BanMMV), cucumber mosaic virus (CMV) and banana bract mosaic virus (BBrMV) have been detected in this crop in south America (Bhat et al. 2016; Reichel et al. 2003; Eiras et al. 2004; Quito-Avila et al. 2013). CMV is one of the most important plant viruses, infecting more than 1000 plant species worldwide (Jacquemond 2012). Both subgroups I and II has been detected in horticultural crops from Argentina (Atencio et al. 1997), however, there are no sequences of any Argentine CMV isolates available in international nucleotide sequence databases. Moreover, no information is known about the status of viruses infecting banana in the North of Argentina, which is the main growing region in this country.

In July 2017, Cavendish banana (*Musa* sp. AAA group) cv. 'Congo' showing mosaic symptoms in leaves was observed (Fig. 1a) and collected in Laguna Naineck, department Pilcomayo, Northeast of Argentina. These symptoms were similar to those induced by viruses in banana crop (Selvarajan 2015).

Symptomatic banana leaf tissues were analyzed by transmission electron microscopy; leaf-dip preparations were made with borate buffer 0.05 M pH 7.0 and stained with 2% uranyl acetate (Sigma-Aldrich, USA) (Kitajima and Nome 1999). The observations revealed the presence of isometric particles ca. 30 nm in diameter (Fig. 1b). In these observations, particles belonging to other virus families were not detected. Leaf extracts obtained from symptomatic leaves were serologically analyzed for CMV using polyclonal and monoclonal antibodies. Firstly by ImmunoStrip® and after, by Triple Antibody Sandwich (TAS-ELISA) test against CMV subgroups I and II

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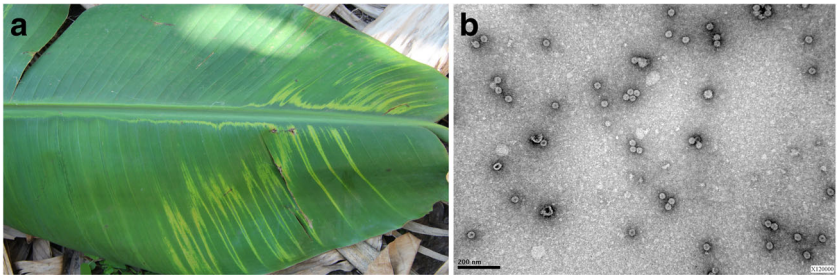

Fig. 1 Mosaic and streak symptoms associated with cucumber mosaic virus infection of banana in Laguna Naineck, Argentina (**a**). Electron micrographs of negatively stained virus particles (~30 nm in diameter) with 2% uranyl acetate (**b**)

(Agdia, USA). Reactions were positive to CMV and CMV subgroup I, respectively.

Leaf extracts obtained from infected banana plant were used to inoculate healthy *Nicotiana glutinosa* plants, which was performed according to Singh et al. (1995). Plants of *N. glutinosa* showed symptoms, similar to those induced by this virus (data not shown). In the naturally infected and experimentally inoculated plants, virus infections were confirmed by RT-PCR.

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. The reactions to amplify the CP gene were performed in two steps. First-strand cDNAs were synthesized using Moloney Murine Leukemia virus reverse transcriptase enzyme (M-MLV) (Promega, USA) according to the manufacturer's instructions and 3'CP as the initial primer (Rizos et al. 1992). The reaction was incubated for 4 min at 99 °C, followed by 60 min of incubation at 42 °C. PCR was carried out using Kapa Hiffi DNA polymerase enzyme (Kapa Biosystems, USA) and primers: 5'CP (5'-CTCGAATT CGGATCCGCTTCTCCGCGAG-3′) and 3'CP (5'-GGCGAATTCGAGCTCGCC GTAAGCTGGATGGAC-3′),

Table 1 Nucleotide (nt) and amino acid (aa) sequence identities of an Argentine cucumber mosaic virus isolate (MH716245) with isolates from other geographic regions

Host	Subgroup	Origin	GenBank	Identities (%)	
				nt	aa
Mertensia virginica	IA	USA	GU362669	98.3	98.1
Anemone sp.	IA	USA	FJ375723	98.3	98.1
Cucumis melo	IA	Serbia	KT270566	98.3	98.6
Canna sp.	IA	Russia	KY595419	98.3	98.1
Solanum lycopersicum	IA	Serbia	KC847071	98.3	98.1
Cucumis melo	IA	USA	D10538	98.1	98.1
Nicotiana tabacum	IA	USA	U20668_	98.1	98.1
Iris sp.	IA	Iran	KM262647	98.1	98.1
Musa balbisiana	IA	DR Congo	KX216873	98.1	98.1
Musa balbisiana	IA	DR Congo	KX216870	98.1	98.1
Musa balbisiana	IA	DR Congo	KX216868	98.1	98.1
Musa acuminata	IA	DR Congo	KX216872	98.1	98.1
Musa acuminata	IA	DR Congo	KX216869	98.1	98.1
Musa acuminata	IA	DR Congo	KX216871	98.1	98.1
Chrysanthemum	IA	Brazil	AY380533	98.0	97.7
Solanum lycopersicum	IB	Italy	Y16926	95.1	97.2
Nicotiana tabacum	IB	China	AB008777	93.8	96.7
Capsicum sp.	IB	Italy	HE962480	93.7	96.7
Musa sp.	IB	Nigeria	KU976478	93.4	95.4
Musa sapientum	IB	Indonesia	AB069971	92.8	96.7
Capsicum sp.	II	Australia	M21464	77.0	82.1
Musa sp.	II	China	AF268598	76.7	81.7
Lactuca saligna	II	USA	AF127976	76.7	81.2
Lupinus angustifolius	II	Australia	AF198103	76.4	81.7

Origin and GenBank accession numbers of the nucleotides sequences of CMV isolates used in phylogenetic analysis are presented

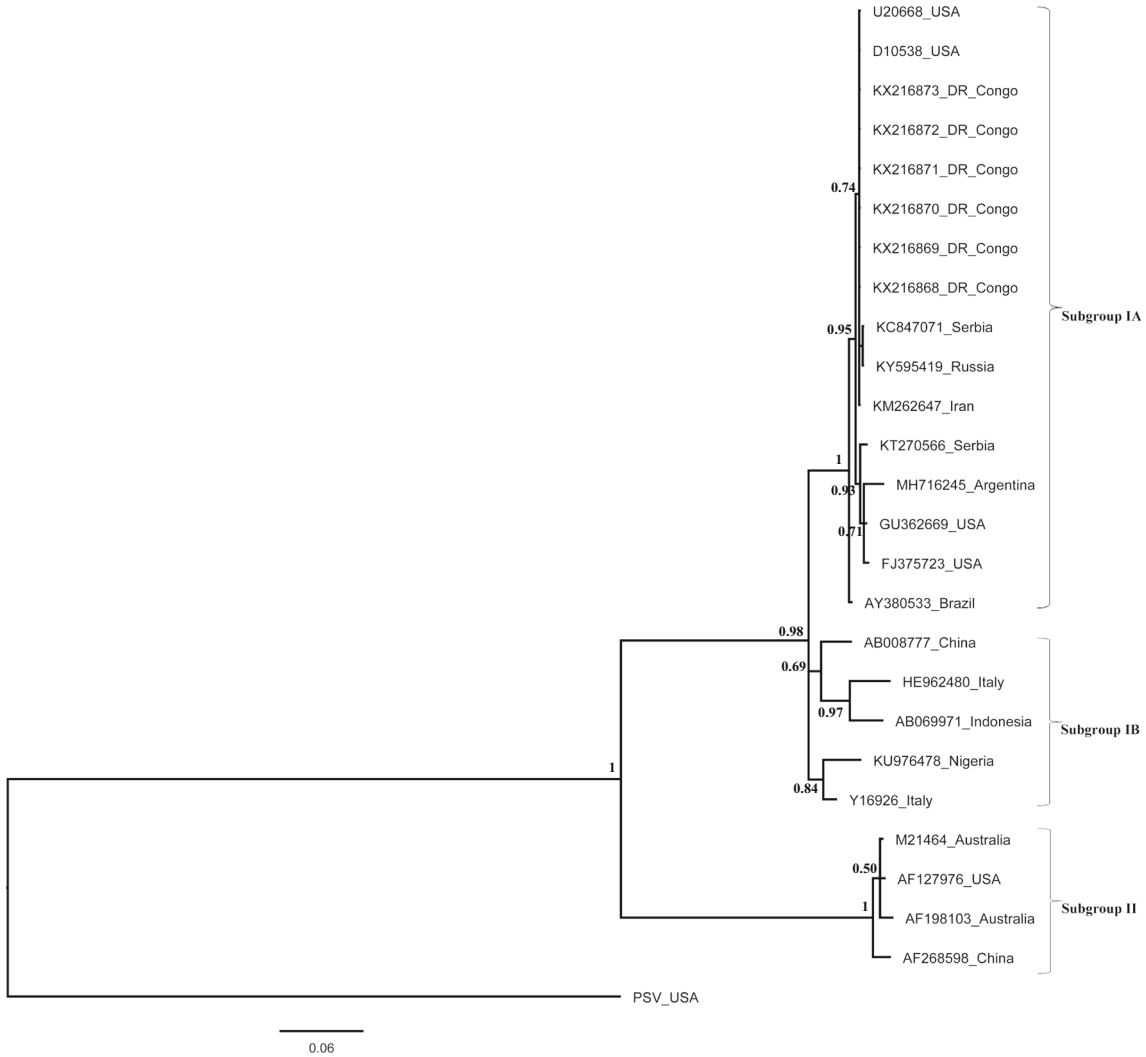

Fig. 2 Majority rule consensus tree obtained from the Bayesian analysis of coat protein nucleotide sequences of an Argentine cucumber mosaic virus isolate (MH716245) and other 25 CMV isolates available at GenBank database. Branch lengths are proportional to genetic distances and branch significance is indicated at nodes for posterior probability values higher than 0.5. For each isolate, the GenBank accession number and geographic origin are indicated. The CP gene sequence of Peanut stunt virus (PSV) (U15730) was used as an outgroup for comparison

which amplifies the 3′ portion of RNA 3 in CMV isolates (Rizos et al. 1992). PCR reactions was performed using 2 μl of cDNA, 1 U of Kapa Hiffi DNA polymerase enzyme (Kapa Biosystems, USA), 300 μM dNTPs each and 0.3 μM of each primers in a final reaction volume of 50 μl. The PCR conditions included an initial incubation for 2 min at 95 °C, followed by 34 cycles of denaturation at 94 °C for 45 s, annealing at 40 °C for 45 s, extension for 1 min at 72 °C and final extension at 72 °C for 10 min.

The products obtained by RT-PCR of ~800 bp (data not shown) were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) and then directly sequenced in both directions at Macrogen Inc., Seoul, Korea. The sequence determined in this study was deposited in international nucleotide sequence databases with Accession number MH716245. Preliminary sequence data analysis of the CP gene was performed with the NCBI blast tool to search for related sequences. CMV complete CP sequences from different origins, with identities greater than 75%, were selected from GenBank database for further analysis. Sequence alignment of CMV isolates was carried out using MUSCLE (Edgar 2004), by mean of SeaView software v. 4.4.2 (Gouy et al. 2009). The model of base substitution was estimated using the jModelTest v. 2.1.9 software (Darriba et al. 2012), according to the Akaike Information Criterion. Phylogenetic trees were obtained by using Bayesian inference in Mr. Bayes 3.2.6 (Ronquist et al. 2012).

The values of the sequence identities between the Argentine CMV isolate and others from different geographic regions and subgroups ranged from 76.4 to 98.3% and from 81.2 to 98.6% at the nucleotides and amino acids levels, respectively (Table 1). According to

literature data, the members of the same subgroup share more than 90% homology (Palukaitis and García-Arenal 2003). In this study, the nucleotide homology between isolates from I and II subgroups was approximately 75.3–78%. Argentine isolate was phylogenetically most related to the members of subgroup IA (≥98% nt homology), which coincides with the sequence homology values reported in the same subgroup (Gallitelli 2000). However, they were distinct from the subgroups IB and II isolates. Members of subgroup II showed the lowest nucleotide homology (76–77%) with the Argentine CMV isolate (Table 1).

Bayesian phylogenetic tree of nucleotide sequences showed three distinct clusters corresponding to subgroups IA, IB and II. Reference CP gene sequences of CMV isolates Tfn for subgroup IB (Y16926), Fny for subgroup IA (D10538) and Q for subgroup II (M21464) were used for subgrouping (Fig. 2). Within subgroup I, the Argentine CMV isolate showed close phylogenetic relationships with isolates from United States of America and Serbia (GU362669, FJ375723 and KT270566). The phylogeny of CMV shows no clear correlation with the provenance of the isolates (Fig. 2), which suggest that CMV world population seems to be thoroughly mixed (Ohshima et al. 2016).

From an epidemiological point of view, the results presented are not only important in Argentina, but also for neighboring countries. The banana producing region of Argentina where the virus was detected is separated only by the Pilcomayo river from the Paraguay. This geographical scenario, linked to the absence of sexual reproduction in vegetative propagated plants and the subtropical conditions that favour the development of vector aphids, enables viruses to establish long-term infections and to be transmitted with high efficiency. Moreover, the occurrence of CMV in banana from Northeast Argentina is possibly associated with the wide host range of this virus in perennial weed and cultivated species and constitutes an alert due to the propagation methods used by regional producers. To our knowledge, this is the first report of the occurrence of CMV infecting banana in Argentina and the unique viral disease detected in banana in this country. In 2018, it was developed the Phytolab technology in Misión Tacaaglé (Formosa province), which estimates the field establishment of in vitro-produced banana plants to restore 200 ha per year. The presence of this virus suggests the monitoring of virus-free propagative materials, which is required for the establishment of new plantations through asexual or in vitro propagation.

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References

Atencio FA, Gracia O, Mendoza EEA, Zandomeni R, Grau O (1997) Detection of both subgroups I and II of cucumber mosaic Cucumovirus and their satellite RNAs on pepper in Argentina. Plant Dis 81:695–695

Bhat AI, Hohn T, Selvarajan R (2016) Badnaviruses: the current global scenario. Viruses 8:177

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:1–19

Eiras M, Boari AJ, Colariccio A, Chaves ALR, Briones MRS, Figueira AR, Harakava R (2004) Characterization of isolates of the Cucumovirus cucumber mosaic virus present in Brazil. J Plant Pathol 86:61–69

Gallitelli D (2000) The ecology of cucumber mosaic virus and sustainable agriculture. Virus Res 71:9–21

Gouy M, Guindon S, Gascuel O (2009) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221–224

Jacquemond M (2012) Cucumber mosaic virus. Adv Virus Res 84:439–504

Kitajima EW, Nome CF (1999) Microscopía electrónica en virología vegetal. In: Docampo DM, Lenardon SL (eds) Métodos para detectar patógenos sistémicos. Instituto de Fitopatología y Fisiología Vegetal (IFFIVE) INTA-JICA, Córdoba, Argentina, p 75–77

Molina NA (2016) La producción de frutas tropicales: panorama mundial y en Argentina. EEA INTA Bella Vista. Hoja de divulgación N° 42:19

Ohshima K, Matsumoto K, Yasaka R, Nishiyama M, Soejima K, Korkmaz S, Ho SYW, Gibbs AJ, Takeshita M (2016) Temporal analysis of reassortment and molecular evolution of cucumber mosaic virus: extra clues from its segmented genome. Virology 487:188–197

Palukaitis P, García-Arenal F (2003) Cucumoviruses. Adv Virus Res 62:241–323

Quito-Avila DF, Ibarra MA, Alvarez RA, Ratti MF, Espinoza L, Cevallos-Cevallos JM, Peralta EL (2013) First report of banana bract mosaic virus in 'Cavendish'banana in Ecuador. Plant Dis 97:1003

Reichel H, Martinez AK, Arroyave JA, Sedano R, Morales FJ, Duterme O, Kummert J, Lepoivre P (2003) First report of banana mild mosaic virus isolated from plantains (Musa AAB) in Colombia. Plant Dis 87:1150

Rizos H, Gunn LV, Pares RD, Gillings MR (1992) Differentiation of cucumber mosaic virus isolates using the polymerase chain reaction. J Gen Virol 73:2099–2103

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542

Selvarajan R (2015) Viral diseases of banana. CAB Reviews 10:1–16

Singh Z, Jones RAC, Jones MGK (1995) Identification of cucumber mosaic virus subgroup I isolates from banana plants affected by infectious chlorosis disease using RT-PCR. Plant Dis 79:713–716

Tapia AC, Fagiani MJ (2015) Argentina: Situación bananera pasado, presente y futuro. III Congreso Latinoamericano y del Caribe de Plátanos y Bananos, Brazil