

Evidence of different phylogenetic origins of two mexican Sugarcane mosaic virus (SCMV) isolates

Evidencia de orígenes filogenéticos diferentes de dos aislamientos mexicanos del virus del mosaico de la caña de azúcar (SCMV)

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Abstract

The molecular analysis of the Sugarcane mosaic virus (SCMV) for coat protein cistron reported in the public GenBank database, revealed the presence of 45 additional nucleotides coding for 15 amino acids in the N-terminal region of the coat protein sequence of the Mexican isolate GU474635. BLAST analysis indicates this particular feature is also present in the coat protein sequence identified with the accession number D00949 reported in the USA in 1991. Phylogenetic analysis of 185 SCMV coat protein sequences reported from Asia, Africa, Brazil and Argentina among others, suggest a putative different phylogeographic origin of the Mexican SCMV isolates. Coat protein sequence from isolate GU474635 is phylogenetically closer to isolates from Brazil and USA, while SCMV coat protein sequences from Germany and Spain are phylogenetically closer to the coat protein from isolate EU091075. Particular features among SCMV isolates from different countries along the American continent, i.e USA, Mexico and Brazil suggest low phytosanitary control in plant material exchange among countries.

Keywords: Coat protein, maize, mosaic virus, phylogeny.

Resumen

El análisis molecular del cistron, que codifica para la proteína de la cubierta del virus del mosaico de la caña de azúcar (SCMV) reportadas en la base de datos del banco de genes (GenBank), reveló la presencia de 45 nucleótidos adicionales que codifican para 15 aminoácidos en la región amino de la secuencia de la proteína de la cubierta del aislamiento mexicano identificado con el número de acceso GU474635. El análisis BLAST indicó que esta característica particular está también presente en el aislamiento D00949, reportado en 1991 en Estados Unidos. El análisis filogenético de 185 secuencias de la proteína de la cubierta de SCMV reportadas de Asia, Africa, Brasil y Argentina, entre otros, sugiere diferentes orígenes filogeográficos de los aislamientos mexicanos. El aislamiento mexicano GU474635 es filogenéticamente más cercano a aislamientos de SCMV de Brasil y de EE.UU., mientras que secuencias de la proteína de la cubierta del virus SCMV reportados en China y Alemania son filogenéticamente más cercanos al aislamiento mexicano EU091075. Las características particulares que comparten aislamientos virales de tres países a lo largo del continente americano, EE.UU., México y Brasil, sugieren un bajo control fitosanitario en el intercambio de material vegetal.

Palabras clave: Filogenia, maíz, proteína de la cubierta, virus del mosaico.

Introduction

Sugarcane mosaic virus (SCMV) is a member of the *Potyvirus* group in the *Potyviridae* family, which can infect different crops including sugarcane, sorghum and maize leading to mosaics, chlorosis and dwarfism (Shukla *et al.*, 1989). Traditionally, SCMV isolates from sugarcane were designated as SCMV races and the ones from maize as MDMV races. However, both, the SCMV races and the MDMV-B races share a lot of common properties and, therefore, MDMV-B was considered a SCMV race (Shukla *et al.*, 1994). These potyvirus that infect sugarcane were included in the SCMV subgroup, which has four different but related species: SCMV, sorghum mosaic virus (SrMV), maize dwarfism mosaic virus (MDMV) and Johnson grass mosaic virus (JGMV). Among these viruses, only SCMV and SrMV infect sugarcane in natural conditions and are considered causal agents of mosaic in this plant being reported in more than 70 countries (Jeffery *et al.*, 1998).

The viral particles of this family are filamentous and are in length between 650 and 900 nm and width between 11 and 13 nm. They have a simple chain of RNA of 10 Kb approximately. SCMV genome is polyadenylated (Adams *et al.*, 2005) and has a VPg protein covalently bound to the 5' end. Genome is surrounded by 200 units of coat proteins (CP) (Chen *et al.*, 2001). The potyviral CP has different functions including aphid transmission, cell to cell movement, systemic movement, genome encapsidation, and regulation of RNA amplification. The amino region of CP has a DAG motif that is highly conserved between *Potyvirus* transmitted by aphids (Dombrovsky *et al.*, 2005). Genetic structure analysis and population variation are critical areas of biology and, in the case of viruses, it is highly relevant for the development of control strategies for diseases and epidemics, and for diagnosis (Jridi *et al.*, 2006; Martin *et al.*, 2006). This has generated an increasing interest in the genic structure of viral populations in the last two decades (Fondong y Chen, 2011; Garcia-Arenal *et al.*, 2001; Ge *et al.*, 2007; Glasa *et al.*, 2011; Holmes, 2003; Jridi *et al.*, 2006; Martin *et al.*, 2006; Moreno *et al.*, 2004; Rommelfanger *et al.*, 2012; Yoshida *et al.*, 2012; Zhang *et al.*,

2011). Understanding the viral genetic stability and the nucleotide composition of different isolates from diverse origins, are key aspects to develop strategies for control of viruses (Moreno *et al.*, 2004; Tan *et al.*, 2004).

In this study were analyzed the nucleotide sequences of 185 CPs reported around the world, aiming to establish the phylogenetic relationship of the two unique sequences from America (Mexico) that have been completed and reported in the GenBank (Isolates 1 and 2 in Table 1). Molecular analyses indicate differences between the American isolates in the amino region of the coat protein. This difference results in two putative SCMV populations with different phylogenetic origin that infect maize in its center of origin and diversification.

Materials and methods

Sequences of the SCMV protein coat and alignment

The CP sequences of the SCMV were searched in the public sequence database known as "GenBank". For the study 206 sequences were selected and are indicated in Table 1. For a more detailed identification, in each accession is indicated the country of origin, recollection and/or publication year and the host if available. The criteria for sequence selection were the presence of the highly conserved motif DAG. All the sequences were aligned deducing their amino acids by ClustalW in the software MEGA v. 4.0 (Kumar *et al.*, 2008), using default parameters. Sequence alignment was manually adjusted if necessary. From the 206 initial sequences, the incomplete or short ones, or the ones that generated problematic gaps for the alignment were ruled out.

With the previous criteria, 21 sequences were eliminated for a total of 185 sequences highlighted in gray in Table 1. Based on the amino acids alignment of the 185 sequences, each sequence was manually adjusted to 747 nucleotides that code for 249 amino acids, counting from the DAG motif until the amino acid consensus sequence **SRTPARAKEA**. The amino acids highlighted in bold are highly conserved in all the sequences. This proce

Table 1. CP sequences of the SCMV from the GenBank used in the analysis. Hos: Host, hospedante, MZ; maíz, SC; caña de azúcar, NA; sin información

	GenBank	PAIS	HOSP	AÑO		GenBank	PAIS	HOS	AÑO		GenBank	PAIS	HOS	AÑO		GenBank	PAIS	HOSP	AÑO
1	EU091075	MEX	MZ	2010	53	AJ491933	AFR	SC	2003	105	AY630923	THAI	SC	2004	157	EF419173	CHN	SC	2007
2	GU474635	MEX	MZ	2010	54	AJ491934	AFR	SC	2004	106	AY639645	CHN	MZ	2004	158	JN021933	CHN	MZ	2011
3	AF006728	NA	NA	1997	55	AJ491935	AFR	SC	2005	107	AY836523	US	MZ	2004	159	EF507708	CHN	SC	2007
4	AF006730	NA	NA	1997	56	AJ491936	AFR	SC	2006	108	AY953351	CHN	NA	2005	160	EF507709	CHN	SC	2007
5	AF006731	NA	NA	1997	57	AJ491937	AFR	SC	2007	109	D00948	AUS	SC	1991	161	EF507710	CHN	SC	2007
6	AF006733	NA	NA	1997	58	AJ491938	AFR	SC	2008	110	D00949	AUS	NA	1991	162	EF507711	CHN	SC	2007
7	AF006734	NA	NA	1997	59	AJ491939	AFR	SC	2009	111	DQ227694	CHN	SC	2005	163	EF507712	CHN	SC	2007
8	AF006735	NA	NA	1997	60	AJ491940	AFR	SC	2010	112	DQ315489	BRA	NA	2005	164	EF507716	CHN	SC	2007
9	AF006736	NA	NA	1997	61	AJ491941	AFR	SC	2011	113	DQ315490	BRA	NA	2005	165	EU196421	ARG	SC	2007
10	AF006737	NA	NA	1997	62	AJ491942	AFR	SC	2012	114	DQ315491	BRA	NA	2005	166	EU196422	ARG	SC	2007
11	AF006738	NA	NA	1997	63	AJ491943	AFR	SC	2013	115	DQ315492	BRA	NA	2005	167	EU196423	ARG	SC	2007
12	AF494510	CHN	MZ	2002	64	AJ491944	AFR	SC	2014	116	DQ315493	BRA	NA	2005	168	EU196424	ARG	SC	2007
13	AJ006199	ALE	NA	1998	65	AJ491945	AFR	SC	2015	117	DQ315494	BRA	NA	2005	169	EU196425	ARG	SC	2007
14	AJ006200	ALE	NA	1998	66	AJ491946	AFR	SC	2016	118	DQ315495	BRA	NA	2005	170	EU196426	ARG	SC	2007
15	AJ006201	ALE	NA	1998	67	AJ491947	AFR	SC	2017	119	DQ315496	BRA	NA	2005	171	EU196427	ARG	SC	2007
16	AJ006202	ALE	NA	1998	68	AJ491948	AFR	SC	2018	120	AJ491985	AFR	SC	2005	172	EU196428	ARG	SC	2007
17	AJ271085	CHN	MZ	2000	69	AJ491949	AFR	SC	2019	121	AJ491986	AFR	SC	2005	173	EU196429	ARG	SC	2007
18	AJ297628	CHN	MZ	2002	70	AJ491950	AFR	SC	2020	122	AY630923	THAI	SC	2005	174	EU196430	ARG	SC	2007
19	AJ310102	CHN	SC	2002	71	AJ491951	AFR	SC	2021	123	AY639645	CHN	MZ	2005	175	EU196431	ARG	SC	2007
20	AJ310103	CHN	SC	2002	72	AJ491952	AFR	SC	2022	124	AY836523	US	MZ	2005	176	EU196432	ARG	SC	2007
21	AJ310104	CHN	SC	2002	73	AJ491953	AFR	SC	2023	125	AY953351	CHN	NA	2005	177	EU196433	ARG	SC	2007
22	AJ310105	CHN	MZ	2002	74	AJ491954	AFR	SC	2024	126	D00950	AUS	SC	2005	178	EU196434	ARG	SC	2007
23	AJ310106	CHN	MZ	2001	75	AJ491955	AFR	SC	2025	127	D00951	AUS	NA	2005	179	EU196435	ARG	SC	2007
24	AJ310107	CHN	MZ	2001	76	AJ491956	AFR	SC	2026	128	DQ227694	CHN	SC	2005	180	EU196436	ARG	SC	2007
25	AJ310108	CHN	MZ	2001	77	AJ491957	AFR	SC	2027	129	DQ315489	BRA	NA	2005	181	EU196437	ARG	SC	2007
26	AJ310109	CHN	MZ	2001	78	AJ491958	AFR	SC	2028	130	DQ315490	BRA	NA	2005	182	EU196438	ARG	SC	2007
27	AJ310110	CHN	MZ	2001	79	AJ491959	AFR	SC	2029	131	DQ315491	BRA	NA	2005	183	EU196439	ARG	SC	2007
28	AJ310111	CHN	MZ	2001	80	AJ491960	AFR	SC	2030	132	DQ315492	BRA	NA	2005	184	EU196440	ARG	SC	2007
29	AJ311168	ESP	NA	2001	81	AJ491961	AFR	SC	2031	133	DQ315493	BRA	NA	2005	185	EU196441	ARG	SC	2007
30	AJ311169	ESP	NA	2001	82	AJ491962	AFR	SC	2032	134	DQ315494	BRA	NA	2005	186	EU196442	ARG	SC	2007
31	AJ421467	CHN	SC	2001	83	AJ491963	AFR	SC	2033	135	DQ315495	BRA	NA	2005	187	EU196443	ARG	SC	2007
32	AJ421468	CHN	SC	2001	84	AJ491964	AFR	SC	2034	136	DQ315496	BRA	NA	2006	188	EU196444	ARG	SC	2007
33	AJ421469	CHN	SC	2001	85	AJ491965	AFR	SC	2035	137	AJ491987	AFR	SC	2006	189	EU196445	ARG	SC	2007
34	AJ438190	CHN	NA	2002	86	AJ491966	AFR	SC	2036	138	AJ491988	AFR	SC	2006	190	EU196446	ARG	SC	2007
35	AJ491917	AFR	SC	2002	87	AJ491967	AFR	SC	2037	139	AY630923	THAI	SC	2006	191	EU196447	ARG	SC	2007
36	AJ491918	AFR	SC	2002	88	AJ491968	AFR	SC	2038	140	AY639645	CHN	MZ	2006	192	EU196448	ARG	SC	2007
37	AJ491919	AFR	SC	2002	89	AJ491969	AFR	SC	2039	141	AY836523	US	MZ	2006	193	EU196449	ARG	SC	2007
38	AJ491920	AFR	SC	2002	90	AJ491970	AFR	SC	2040	142	AY953351	CHN	NA	2006	194	EU196450	ARG	SC	2007
39	AJ491921	AFR	SC	2002	91	AJ491971	AFR	SC	2041	143	D00952	AUS	SC	2006	195	EU196451	ARG	SC	2007
40	AJ491922	AFR	SC	2002	92	AJ491972	AFR	SC	2042	144	D00953	AUS	NA	2006	196	EU196452	ARG	SC	2007
41	AJ491923	AFR	SC	2002	93	AJ491973	AFR	SC	2043	145	DQ227694	CHN	SC	2006	197	EU196453	ARG	SC	2007
42	AJ491924	AFR	SC	2002	94	AJ491974	AFR	SC	2044	146	DQ315489	BRA	NA	2006	198	EU196454	ARG	SC	2007
43	AJ491925	AFR	SC	2002	95	AJ491975	AFR	SC	2045	147	DQ316232	CHN	SC	2006	199	EU196455	ARG	SC	2007
44	AJ491926	AFR	SC	2002	96	AJ491976	AFR	SC	2046	148	DQ316235	CHN	MZ	2006	200	EU650180	IND	SC	2008
45	AJ491927	AFR	SC	2002	97	AJ491977	AFR	SC	2047	149	DQ316236	CHN	SC	2006	201	NC003398	CHN	MZ	2000
46	AJ491928	AFR	SC	2002	98	AJ491978	AFR	SC	2048	150	DQ316238	CHN	SC	2006	202	X98165	ALE	NA	1996
47	AJ491929	AFR	SC	2002	99	AJ491979	AFR	SC	2049	151	DQ316239	CHN	MZ	2006	203	X98166	ALE	NA	1996
48	AJ491930	AFR	SC	2002	##	AJ491980	AFR	SC	2050	152	DQ866744	IND	SC	2006	204	X98167	ALE	NA	1996
49	AJ491931	AFR	SC	2002	##	AJ491981	AFR	SC	2051	153	DQ866745	IND	SC	2006	205	X98168	ALE	NA	1996
50	AJ491932	AFR	SC	2002	##	AJ491982	AFR	SC	2052	154	DQ866746	IND	SC	2006	206	X98169	ALE	NA	1996
51	AJ491983	AFR	SC	2053	##	DQ866741	IND	SC	2006	155	EF066741	IND	SC	2006					
52	AJ491984	AFR	SC	2054	##	EF066739	IND	SC	2006	156	EF419171	CHN	SC	2007					

ture pretended a better alignment to get more trustable phylogenetic trees.

Phylogenetic trees

Phylogenetic trees were constructed using the Neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) in the MEGA program. Sequence divergence was estimated by Kimura's two parameters method (Kimura, 1980) and the phylogenetic trees were visualized with 'tree explorer' in MEGA 4.0. To estimate the confidence of the branching patterns of the phylogenetic trees a resampling value with 1000 replicates was used. Phylogenetic trees generated in MEGA were exported in PDF format and edited in Canvas 10 in Mac OSX 10.6.8.

Results and discussion

Genomic structure

Initial alignment of the deduced amino acids of the complete sequences of the SCMV coat protein allowed the identification of sequences reported in Brasil, USA and Mexico, with a total of 328 amino acids and 15 additional amino acids in comparison with most of the CP sequences. CP sequence of the isolate identified with accession number GenBank GU474635, reported in Mexico, has a total of 984 nucleotides compared with the sequence of the same genomic region of the EU091075 isolate, Mexican as well, with 939 nucleotides that code for 313 amino acids. The estimated molecular weight of the CP from EU091075 is 33.82kDa while for GU474635 is 34.71kDa. The similarity of both sequences in the CP region is 88.3%. The biological reason of the extra amino acid sequence found in some SCMV isolates could vary. The variable region of the CP of *Potyvirus* is needed for aphid transmission and systemic infection and, is important for virus adaptation to the host. The specificity for viral transmission via vectors is defined by the capacity of a vector to transmit certain viruses but not others (Dombrovsky *et al.*, 2005). In the *Potyvirus* case, transmission depends on the presence of a helper component that interacts with the CP amino terminus (Dombrovsky *et al.*, 2005). The interaction specificity between CP and HC

was characterized in vitro with the tobacco vein mottling virus (TVMV) by protein-protein interaction assays. HC interacts with CP virions or monomers coming from the TVMV transmitted by aphids, but not for TVMV that is not transmitted by aphids. In *Potyvirus*, HC interaction happens with the CP amino terminus including the DAG motif (Blanc *et al.*, 1997), and the amino acids of the amino region close to the DAG motif affect the aphid transmission. This means that the context in which the DAG motif is located plays an important role determining the transmission efficiency of *Potyvirus* by aphids (Lopez-Moya *et al.*, 1999). Recent studies have suggested a role for the CP amino region in recognizing different HC from viruses that infect different hosts (Dombrovsky *et al.*, 2005). In this context is valid to think that, the variation in amino acid number and type close to the DAG motif in the SCMV isolates is caused by the virus specificity for some vectors from the specific regions where they were sampled through the CP and HC interaction. In the other side, it is known that host specificity determinants could be found on the amino region of CP (Salvador *et al.*, 2008), therefore it is possible to suggest that the variability found in this region for the SCMV Mexican isolates could be due to host specificity. Differences in the amino terminus have been also determinant to be used as a molecular criterion to discriminate genera and species in the *Potyviridae* family (Adams *et al.*, 2005).

Analysis of nucleotide sequences alignment

To determine the phylogenetic relationship between the SCMV Mexican isolates, a sequence comparison was done between their CP and the CP sequences of SCMV reported around the world that are publicly available in GenBank <http://www.ncbi.nlm.nih.gov/genbank/>. The search and comparison was performed using BLAST from the National Center of Biotechnological Information (NCBI), <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. The result of the comparison and analysis in BLAST with the complete CP sequence of the Mexican isolate GU474635 indicates that the more related sequence is the one identified with accession number D00949 (Frenkel *et*

al., 1991), with a 95% similarity in their nucleotide sequences (E-value 0.0). The analysis indicates that the same sequences have nucleotide similarities of 87% (E-value 0) with CP sequences of SCMV from Brazil identified with accession numbers DQ315492, DQ315498, DQ315496, DQ315495, DQ315494, DQ315490 and DQ315489, and a similarity of 86% (E-value 0.0) with CP sequences of SCMV also from Brasil identified with accession numbers DQ315493 and DQ315491.

In Frenkel (1991) is reported for the first time, an 'unexpected sequence diversity' in CP of SCMV and MDMV-B isolates in Iowa, USA, that consisted on an amino acids duplication in the amino terminus of MDMV-B. D00949 isolate was obtained from sweet corn fields in Iowa and was designated as Iowa 66-188 (ATCC-PV53) (Hill *et al.*, 1973). In this way, the CP sequence of the Mexican SCMV isolate GU474635 is highly related with USA isolates. These isolates, together with the Brazilian ones, have the longest CP of SCMV found on databases; it has 984 nucleotides possibly coming from an amino acids duplication event (Frenkel *et al.*, 1991). Since there is not available information of other cistrons in the USA and Brazilian isolates, it is not possible to determine whether the Mexican isolates are related with the rest of the genome. The presence of this particular nucleotide fragment in the SCMV isolates reported in different countries along America suggest, first, possible recombination events between isolates; second, long distance transport of infected material/viral isolates; and third, the need of adequate quarantines for germplasm introduction that can have new viral variants. Molecular ecology has revealed that together with recombination, synergism between viral species, new vectors and host adaptation, long distance movement is one of the causal factors of severe viral tropical diseases emergence (Fargette *et al.*, 2006). The restriction in germplasm movement between countries is not that strict, creating the need of increasing safety measures to prevent the introduction of new viral variants that can cause disease risks.

Phylogenetic relationships

The phylogenetic tree generated by the alignment of 185 CP sequences of SCMV (Figure 1) reported in different continents, cluster the Mexican, Brazilian and USA D00949 isolates in the same clade with an acceptable resampling value of 69%. This result suggests that the isolates D00949 from USA, GU474635 from Mexico and the Brazilian ones can have a common genetic origin. On the other side, the EU091075 isolate, also from Mexico, is more related to sequences coming from Germany and China with nucleotide similarities of 92.5% and 92.4%, respectively, according to the pairing comparison results using the Martínez-NW algorithm (Martínez, 1983) implemented in the MegAlign program of the DNASTAR software. Differences in the nucleotide composition of the CP sequences of Mexican SCMV suggest the presence of at least two different SCMV genetic groups infecting maize.

In the phylogenetic tree in Figure 1 is appreciated how SCMV is mainly clustered according to the host from which it was obtained, in this case maize or sugarcane. In the tree there are two main groups, one with the SCMV isolates from sugarcane reported in Africa, China, Argentina and India; and the other group clusters mainly SCMV isolates from maize with some clades with isolates from sugarcane.

Analysis of amino acid alignment

The CP sequence alignment of Mexican SCMV shows that amino acid sequence differences are localized in the amino region where two gaps are formed for the GU474635 isolate (Figure 2A). CP sequence alignment of D00949 and GU474635 isolates did not generate any gap, as expected for their similar length and sequence similarity (Figure 2B).

The comparative analysis of the two previous sequences revealed that the CP sequence of the Mexican isolate GU474635 presents the amino acid duplication previously reported by Frenkel (Frenkel *et al.*, 1991) that has not been reported for other sequences. Some differences can be appreciated in the region of study possibly due to mutations. In the position 41 there is an amino acid change

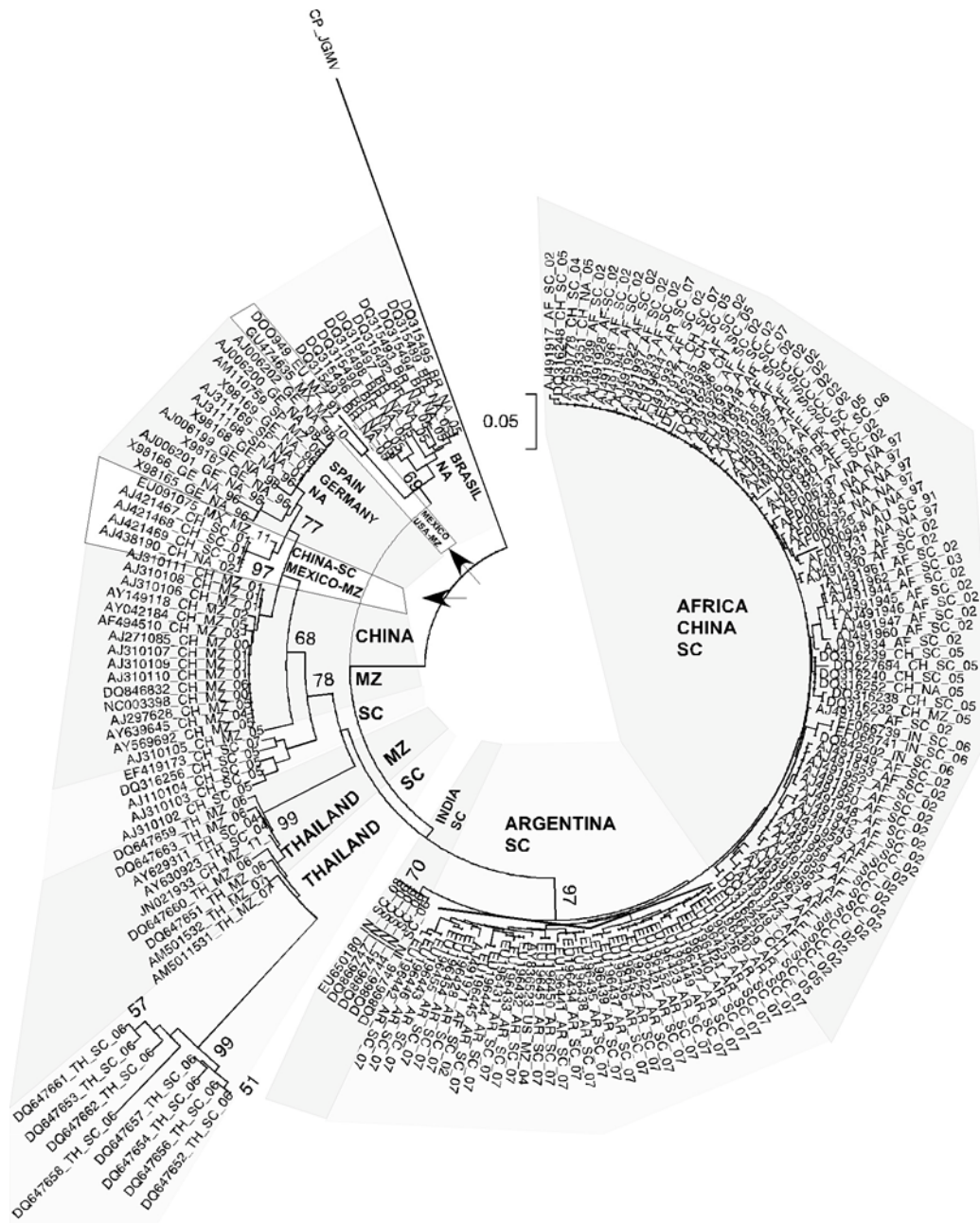


Figure 1. Phylogenetic tree with 185 CP sequences from SCMV with different geographic origins and hosts. Mexican isolates clustered in different clades are indicated by arrows. Each taxon is defined with accession number, , país, hospedante y fecha de recolección/publicacion. SC = Caña de azúcar. MZ = maíz, NA = no disponible.

(A → T (GCT →ACT), transition), in the position 53 there is a change in G → T (GGC →AGT/C, transition) and, in the position 56 there is the T→A change (ACT →GCT, transition) (Figure 2B). Finally, the CP alignment of the Mexican SCMV and the isolate generates two gaps of 15 amino acids in total, one bet-

between the amino acids 22 – 32 and other between amino acids 74 – 77 of the EU091075 isolate (Figure 2C). This result confirms the higher relation between the CP of the isolates GU474635 and D00949.

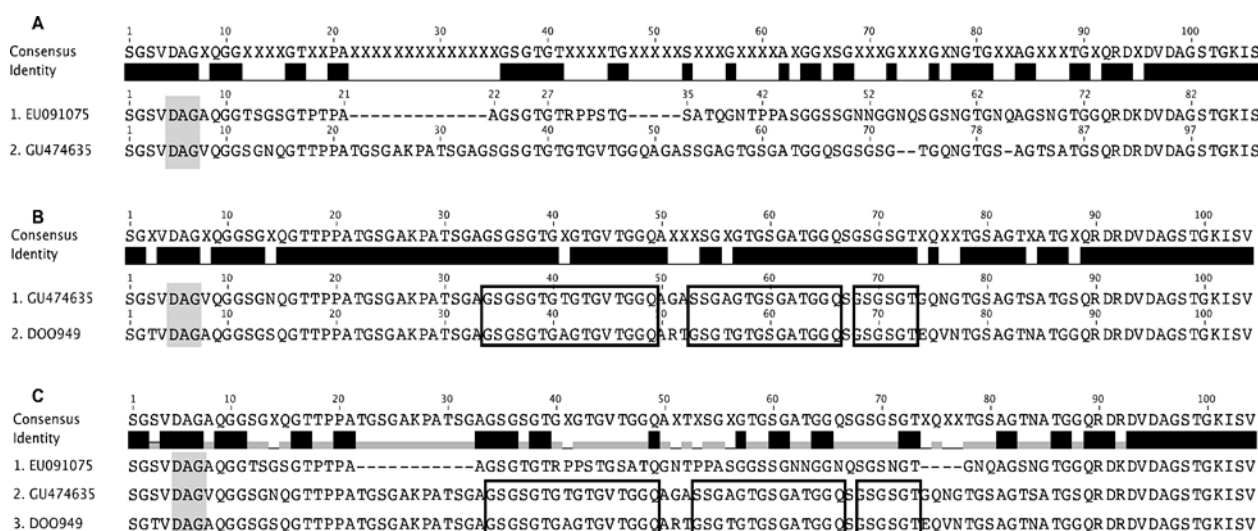


Figure 2. Alignment of CP amino acid sequences of SCMV. The highly conserved DAG motif is indicated in grey boxes. **A.** Alignment of CP sequences of SCMV Mexican isolates. Black boxes indicate the amino acids duplication reported by Frenkel in the D00949 isolate. **B.** Alignment of CP sequences of GU474635 D00949 from USA. **C.** Alignment of CP sequences of SCMV isolates from Mexico and USA.

Conclusions

- The present study reveals the different phylogenetic origins of SCMV isolates from the same country and, the close relation of one of them with isolates from other countries, indicating a low restriction in germplasm movement. Therefore, it arises the need of improving safety measures to prevent introduction of new viral variants that can cause disease risk between countries.
- The difference in nucleotide composition of the Mexican SCMV isolates suggests the presence of at least two viral strains infecting corn in this country.
- There is only a report of two partial sequences of SCMV from Colombia infecting *Elaeis guineensis* (alternative host) (accession number AY072882 and AY072881). The lack of information about this virus, which in Colombia affects mainly crops in the Valle del Cauca and Andean regions, does not allow yet neither the determination of its phylogenetic relation with other isolates, nor the development of control strategies based on biotechnological approaches.

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