Article

Presence of Sugarcane yellow leaf virus (SCYLV) in sugarcane (*Saccharum* spp.) isolated from Colima

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Abstract

Sugarcane is one of the important industrial crops worldwide and is affected by various viral diseases, including the Sugarcane yellow leaf virus (SCYLV). In Mexico, this virus has been detected based on symptomatology and °Brix; however, the diagnosis based on these parameters is not conclusive. The objective of the study was to detect the presence and distribution of SCYLV in sugarcane areas of western Mexico (Colima, Jalisco and Nayarit) and to determine the phylogenetic origin of a Colima isolate. The work was developed in the Tecoman Experimental Field during 2013-2014. Total RNA was isolated from collected leaves and RT-PCR was performed with oligonucleotides specific for SCYLV. A total of 233 samples were analyzed and the incidence of SCYLV carrier plants was 14.6%, affecting the hybrids CP 72-2086, Mex 69-290 and Atemex 96-40. The BLAST analysis showed that the partial CP sequence of Colmex-317 (512 bp) is homologous with sequences from Brazil, China, India, Kenya and USA and also shares identity percentages higher than 99% with other sequences from several countries. The phylogenetic analysis of Colmex-317 with partial sequences and complete genomes of SCYLV isolated from different parts of the world revealed that the Mexican isolate belongs to the Brazilian genotype (BRA) and was grouped with sequences from Brazil, China, Kenya and South Africa. However, it is necessary to evaluate a larger number of isolates and longer sequences to determine if the BRA genotype is the only one present in Mexico.

Keywords: *Saccharum* spp., RNA, RT-PCR, SCYLV.

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Introduction

Viruses cause the most important diseases in sugarcane worldwide, causing large epidemics and losses of enormous proportions. Sugarcane yellow leaf virus (SCYLV), the causative agent of yellow leaf syndrome (YLS), is among the most economically important viral pathogens worldwide for the cultivation of sugarcane. The SCYLV is a member of the Luteoviridae family, in which are located those viruses that cause symptoms of yellowing in host plants that infect, including sugar cane (Rott *et al*., 2008; Girard *et al*., 2012). The characteristic symptom caused by the SCYLV in sugarcane is an intense yellowing in the central vein of the infected leaves, which varies according to the variety and environmental factors (Lockhart and Cronje, 2000). In varieties affected by SCYLV, yield losses of 40-50% have been reported (Vega *et al*., 1997), while in asymptomatic plants with this same virus the losses are 10-30% (Lehrer and Komor, 2008; Lehrer *et al*., 2010). Therefore, this virus is considered in the breeding programs of several sugarcane countries, mainly Brazil, in which the selection and elimination of susceptible clones followed by pathogenicity tests have become routine procedures (Gonçalves *et al*., 2012).

The SCYLV was detected for the first time in Hawaii in 1988 and in Brazil in 1990 (Vega *et al*., 1997; Schenck *et al*., 2001). This virus has been reported in the sugarcane producing areas in several countries. The SCYLV was responsible for drastic economic losses in southeastern Brazil at the beginning of 1990 and is currently the main phytosanitary problem in the breeding programs of that country (Gonçalves *et al*., 2012). In Mexico, the symptoms of YLS were observed for the first time in the year of 1996 affecting the variety CP 72-2086 in the community of La Margarita, Oaxaca. That same year its presence was reported in the same variety in Chiapas, Colima and Veracruz.

It was also detected in Sinaloa affecting the variety Mex 57-473 (Flores, 1997). However, the diagnosis of the disease in Mexico has been based on symptoms typical of the disease associated with YLS and °Brix of the leaf rachis. This is not conclusive because yellowing is a very general symptom of several diseases and deficiencies of micro and macro nutrients, in addition to the pattern of coloration depends on variables such as genotype and environmental conditions (Lockhart and Cronje, 2000).

On the other hand, the levels of the percentage of °Brix are also affected (increasing or decreasing) by the action of some diseases (Vasconcelos *et al*., 2009; Tiwari *et al*., 2012), as well as by chemical elements such as potassium (K), which has a key function in the synthesis of sugars and its translocation in sugarcane stalks, thus increasing the levels of Pol and °Brix (Aucatoma *et al*., 2015). Therefore, it is not reliable to issue diagnoses based on these parameters. The most used and reliable SCYLV detection methods are based on the molecular technique of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and are currently the most used by sugarcane breeding programs in various parts of the world (Xie *et al*., 2009; Joomun and Dookun, 2010; Aday *et al*., 2014; Lin *et al*., 2015).

With respect to the phylogenetic origin of SCYLV, to date 10 different genetic groups have been determined: BRA (Brazil), CHN1, CHN2, CHN3 (China), COL (Colombia), CUB (Cuba), HAW (Hawaii), IND (India), PER (Peru) and REU (Reunion) based on the analysis of the genetic diversity of their genome using partial sequences and complete genomes (Moonan and Mirkov, 2002; Abu Ahmad *et al*., 2006; ElSayed *et al*., 2011; Gao *et al*., 2012; Wang *et al*., 2012; Chinnaraja *et al*., 2013; Lin *et al*., 2015). The genetic diversity of SCYLV populations suggests the existence of large variations among virus isolates, which can affect the degree of virulence, transmission and severity of symptoms associated with SCYLV. Therefore, the objective of this study was to detect the presence and distribution of SCYLV in sugarcane areas of western Mexico (Colima, Jalisco and Nayarit) and to determine the phylogenetic origin of a Mexican isolate.

Materials and methods

Vegetal material

Foliar samples of various varieties of sugarcane commercially planted in the states of Colima, Jalisco and Nayarit were collected during the years 2013-2014. The plants presented symptoms typical of YLS or were asymptomatic. The samples were wrapped in plastic bags and these in coolers with thermal refrigerants to transfer them to the plant biotechnology laboratory of the Tecoman Experimental Field of INIFAP located at km 35 of the Colima-Manzanillo highway in Colima.

Purification, quantification and determination of RNA purity and integrity

Approximately 200 mg of foliar tissue were sprayed with liquid nitrogen until a fine powder was obtained, which was deposited in 1.5 mL tubes and homogenized with 500 µL of Tripure (Roche). The manufacturer's recommendations for the extraction of total RNA were followed. Finally, the obtained RNA was resuspended in RNase-free water (0.01% DEPC) and stored at -70 °C. The quantification was performed with a NanoDrop spectrophotometer (Thermo Scientific) using 1 µL of the extracted total RNA and the ratios of $A_{260/280}$ and $A_{230/280}$ were measured to determine its purity. The integrity of the RNA was corroborated by electrophoresis in 1% agarose gel with TAE 1X buffer and visualized with ethidium bromide (BrEt) in a transilluminator with ultraviolet light (UVP).

Positive control and detection of SCYLV by RT-PCR

The reverse transcription (RT) reaction of the total RNA was carried out using the 'Reverse Transcription System' kit (Promega) according to the manufacturer's instructions. The resulting cDNAs were used as a template for PCR amplification with the set of oligonucleotides SCYLV-F/SCYLV-R described by Xie *et al*. (2009). The final volume of the reaction mixture was 25 µL, containing 12.5 µL of REDTaq® ReadyMixTM (SIGMA-ALDRICH), 1 µM of each oligonucleotide, 3 µL of cDNA and molecular grade H_2O. The reaction mixture was incubated in a thermal cycler (Labnet) with the following program: a cycle of 50 °C for 30 min and 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min.

The electrophoresis was performed on a 1% agarose gel with TAE 1 X buffer and 12.5 µL of the PCR products were loaded, the samples were run with a voltage of 120 V. Finally, the gels were stained with BrEt and visualized in a transilluminator with UV light (UVP) for the analysis of results. In order to have a positive control for the detection of the virus, the PCR product of 512 bp corresponding to a sample from a locality of Tecoman, Colima was purified from the agarose gel with the kit 'QIAquick Gel Extraction' (QIAGEN).

The fragment was cloned with the 'pGEM-T Easy' system (Promega) and transformed into *E. coli* JM109 cells (Promega), which were previously made competent with $CaCl_2$ according to Riley *et al*. (2008). The check of the insert cloned in the vector was made by PCR and restrictive analysis. The extraction of plasmid DNA from the recombinant colonies that was used as a template for the PCR was carried out according to Engebrecht *et al*. (2001), while the restriction enzymes EcoR I and Not I were used for the digestion reaction.

Sequencing, editing and analysis of the sequence

The plasmid DNA of the fragment cloned in *E. coli* was sequenced in an ABI PRISM 310 Genetic Analyzer sequencer in both directions by the termination method with the Big Dye (Applied Biosystems). The editing and assembly of the sequences 'forward' and 'reverse' was done with the CLC Main Workbench 7.0.3 program (https://www.qiagenbioinformatics.com). Finally, the similarity of the obtained sequences against those reported for SCYLV was compared in the database of the National Center for Biotechnology information (NCBI) using the tool 'Basic Local Alignment Searh Tool' (BLAST). The sequence was deposited in the NCBI database.

Phylogenetic analysis

The bioinformatic analyzes were performed with the CLC Main Workbench software version 7.0.3. The BLAST analysis was carried out in NCBI with the SCYLV sequence named ColMex-317 (NCBI: KT334298.1). Complete sequences with the highest percentages of identity were selected and downloaded with ColMex-317 and other sequences from the same database with different geographical origins were used. Subsequently, the multiple sequence alignment tool was used with the MUSCLE algorithm and the maximum likelihood and UPGMA method was used to construct the phylogenetic tree (Edgar, 2004). A test model was used to determine the most appropriate nucleotide substitution model for the data and Kimura 80 was selected. The bootstrap analysis consisted of 1 000 replicates and the CP region of the PRSV was added as an external group (NCBI: AJ012650.1). Finally, the phylogenetic tree was edited with the TreeGraph 2 software.

Results and discussion

The characteristic symptom of intense yellowing in the midribs of sugarcane leaves, associated with the presence of SCYLV, was found in most samples positive for the virus (Figure 1). However, some samples with severe symptoms, similar to those shown in Figure 1, were negative for SCYLV by RT-PCR, which coincides with that reported by Wang *et al*. (2012), who obtained 129/634 positive samples to the SCYLV, of which 96 and 33 were of symptomatic and asymptomatic plants, respectively.

Figure 1. Symptoms of yellow leaf syndrome (YLS) in sugarcane plants. Symptomatology characteristic of intense yellowing in central veins associated with SCYLV is observed.

The above suggests that infection with the virus only has a certain degree of association with the symptoms of yellowing in the field, since this symptom is very general for several diseases of viral origin that affect plants. In sugar cane, the deficiencies of micro and macro nutrients, generate symptoms of yellowing (McCray *et al*., 2006), which could be similar to those caused by the SCYLV. In addition, the pattern of coloration also depends on variables such as genotype and environmental conditions (Lockhart and Cronje, 2000).

On the other hand, the early detection of SCYLV in hybrids of *Saccharum* spp. it is possible, by means of molecular techniques, because there can be detectable levels of the virus in asymptomatic stages (Viswanathan *et al*., 2009). In this study, RT-PCR products subjected to agarose gel electrophoresis that showed the amplification of ~512 bp fragments of SCYLV were considered positive for the virus (Figure 2). Of the 233 foliar samples analyzed, 34 tested positive for the virus, representing an incidence of 14.6%. The SCYLV was presented in the three Pacific states of Mexico that covered the present study (Table 1).

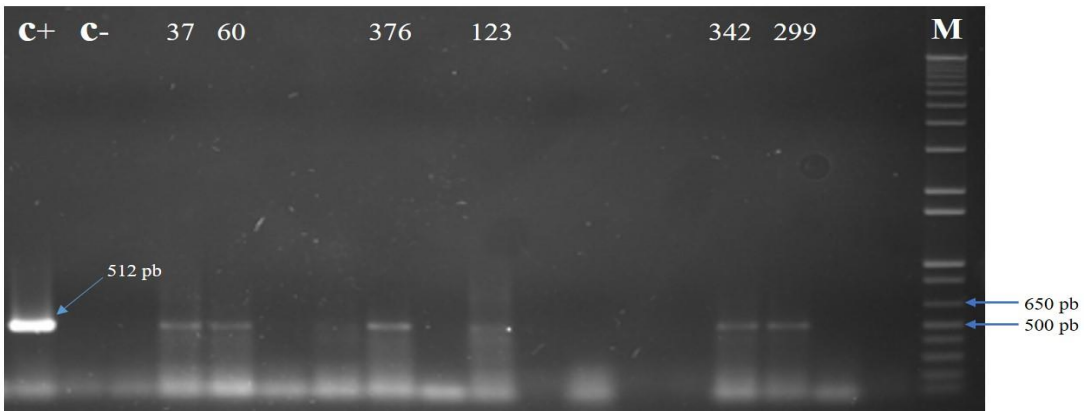

Figure 2. Detection of Sugarcane yellow leaf virus (SCYLV) by RT-PCR in samples of sugarcane from three states of the Mexican Pacific. C+= positive control; C-= negative control; M= 1 Kb plus molecular weight marker (Invitrogen). The numbers correspond to different samples positive to the virus.

Table 1. Relationship of sugarcane samples positive to SCYLV by RT-PCR in the sugarcane region of Western Mexico.

Num.	Sample	State	Municipality	Georeferenced	Variety
1	347	-	-	-	*Saccharum* spp.
2	346	-	-	-	Colmex 95-27
3	2	Colima	Tecoman	N 18°56’06.52” W 104°00’03.54”	*Saccharum spp.*
4	37	Colima	Tecoman	N 18°48’33.23” W 103°50’37.89”	Mex 69-290
5	148	Colima	Tecoman	N 18°52’36.24” W 103°52’1.49”	CP 72-2086
6	336	Colima	Tecoman	N 18°52’36.24” W 103°52’1.49”	CP 72-2086
7	27	Colima	Tecoman	N 18°52’36.24” W 103°52’1.49”	CP 72-2086
8	58	Colima	Tecoman	N 18°52’36.24” W 103°52’1.49”	CP 72-2086
9	60	Colima	Tecoman	N 18°52’36.24” W 103°52’1.49”	CP 72-2086
10	318	Colima	Tecoman	N 18°58’27.53” W 103°51’17.04”	CP 72-2086
11	325	Colima	Tecoman	N 18°58’27.53” W 103°51’17.04”	CP 72-2086
12	380	Colima	Tecoman	N 18°58’27.53” W 103°51’17.04”	CP 72-2086
13	409	Colima	Tecoman	N 18°58’27.53” W 103°51’17.04”	CP 72-2086
14	312	Colima	Tecoman	-	*Saccharum* spp.
15	317	Colima	Tecoman	N 18°58’27.53” W 103°51’17.04”	CP 72-2086
16	376	Colima	Tecoman	N 18°58.455’ W 103°51.285’	CP 72-2086
17	401	Colima	Tecoman	N 18°57’47.88” W 103°50’15.24”	*Saccharum* spp.
18	405	Colima	Tecoman	N 18°57’47.88” W 103°50’15.24”	*Saccharum* spp.
19	123	Jalisco	Zapotiltic	N 19°38’42.58” W 103°24’31”	Atemex 9640
20	138	Jalisco	Zapotiltic	N 19°38’42.58” W 103°24’31”	Atemex 9640
21	340	Jalisco	La Huerta	N 19°31’07.39” W 104°32’11.61”	Atemex 96-40
22	342	Jalisco	Cuautitlan	N 19°26’49.58” W 104°24’10.45”	*Saccharum* spp.
23	344	Jalisco	Cuautitlan	N 19°26’54.74” W 104°23’40.70”	Mex 79-431
24	349	Jalisco	El Grullo	N 19°47’44.20” W 104°13’38.63”	Atemex 96-40
25	248	Jalisco	Zapotiltic	N 19°38’42.58” W 103°24’31”	Atemex 9640
26	281	Jalisco	-	-	Atemex 96-40
27	348	Jalisco	Autlan	-	Mex 69-290
28	217	Nayarit	Jala	N 21°05’08.59” W 104°25’56.82”	Mex 69-290
29	299	Nayarit	Santa María del Oro	N 21°20’23.8” W 104° 34’55.1”'	Mex 69-290
30	169	Nayarit	Xalisco	N 21°27’50.1” W 104° 53’11.4”	*Saccharum* spp.
31	176	Nayarit	Tepic	N 21°27’17.7” W 104° 49’09.2”	*Saccharum* spp.
32	191	Nayarit	Santa Maria del Oro	N 21°15’33.4” W 104° 29’54.3”	*Saccharum* spp.
33	192	Nayarit	Santa Maria del Oro	N 21°15’33.4” W 104° 29’54.3”	*Saccharum* spp.
34	306	Nayarit	-	-	CP 89-2143

In Colima, it was detected mainly affecting the variety CP 72-2086 in Tecoman, while in Jalisco, the virus was detected in Autlan, El Grullo, La Huerta, Cuautitlan and Zapotiltic, affecting Mex 79-431, Mex 69-290 and Colmex 95-27. Finally, in Nayarit the study area comprised the municipalities of Jala, Santa María del Oro, Tepic and Xalisco, where the SCYLV was found affecting the varieties Mex 69-290 and CP 89-2143. This is the first report based on molecular methods for the detection of SCYLV in commercial sugarcane crops in Mexico.

In Cuba, the distribution of SCYLV was determined in different provinces. It is analyzed 525 samples from 35 zones (348 ha), the virus was detected by tissue immunoimpression and RT-PCR, in most of the sampled areas the incidence of symptoms associated with this disease was high (82.29%) (Aday *et al*. 2014). In China, Gao *et al*. (2012), evaluated the distribution of the virus in the main provinces of that country planted with sugar cane in 22 hybrids of *Saccharum* spp. who showed symptoms of YLS using molecular techniques (RT-PCR, qRT-PCR) and serology (TBIA and DAC-ELISA). Of the 22 samples analyzed, 19 were positive for the virus by RT-PCR, TBIA and/or DAC-ELISA, while with the qRT-PCR technique all samples were positive, demonstrating the high sensitivity of qRT-PCR in comparison with RT-PCR and serological techniques to detect pathogens. In another study Wang *et al*. (2012) analyzed samples of sugarcane from the southern part of China to detect the presence of SCYLV.

The expected products of 1.3 kb were amplified in 129/634 (20.3%) individuals by RT-PCR and the virus was present in 6 provinces. Finally, also in China, 332 leaf samples were analyzed to detect the virus by RT-PCR and an incidence of 24-38% was observed, depending on the geographical area (Lin *et al*., 2015). These works show that SCYLV has been detected mainly by molecular techniques, not only in China and Cuba as described, but in various countries of the world such as Argentina, Brazil, India and Kenya (Scagliusi *et al*., 2009; Filippone *et al*., 2010; Lin *et al*., 2015; Amata *et al*., 2016).

The wide distribution of SCYLV in different sugarcane areas of the planet may be due to the form of reproduction of sugarcane stake seed, where the transfer of germplasm between the sugarcane regions of a country to another, sometimes gives way uncontrolled, transporting the pathogenic burden to other regions. Also, the presence of aphid vectors is another important factor for the dispersion of this virus (Figueredo *et al*., 2004).

In relation to the phylogenetic origin of the Mexican isolate, the ColMex-317 sequence was deposited in the NCBI database with the record KT334298. It was determined that the 512 bp sequence corresponds to a partial region of the capsid protein (CP) gene of SCYLV encoded by ORF3. The BLAST analysis indicated that the ColMex-317 sequence of 512 bp is homologous (100% identity) with sequences reported from Brazil, China, India, Kenya and the USA, and shares a high percentage of identity with other sequences of diverse geographic origins.

The results of the phylogenetic analysis performed with the ColMex-317 fragment and other SCYLV sequences (Table 2) with which it shares 96.29-100% identity is shown in Figure 3. The formation of five groups is observed, in which distinguish seven of the ten genotypes reported for SCYLV: HAW, PER, BRA, REU, COL, CHN1 and CHN2 (Moonan and Mirkov, 2002; Abu Ahmad *et al*., 2006; ElSayed *et al*., 2011; Gao *et al*., 2012; Wang *et al*., 2012; Chinnaraja *et al*., 2013; Lin *et al*., 2015). Sequences from Cuba, India and CHN3 were not considered. ElSayed *et al*. (2011) reported the formation of three main groups: BRA, HAW-PER and REU using different phylogenetic trees constructed with fragments of different lengths and complete genomes.

Table 2. Partial and complete sequences of the ORFs and complete genomes of SCYLV obtained from the NCBI database and used in this study for phylogenetic analysis. The CP sequence of the PRSV is included as an external group.

Name of the isolate	Hybrid	Geographical origin	Access NCBI	Fragment size (bp)	Genome portion
Aus1	VMC71-238	Australia	AJ491255.1	672	ORF5, ORF3 and ORF4 partial
BRA1	*Saccharum* spp.	Brazil	AJ582772.1	555	ORF3 and ORF4 partial
BRA-YL1	SP71-6163	Brazil	AM072750.1	5 612	Complete genome
Braz1	SP77-5181	Brazil	AJ491257.1	672	ORF5, ORF3 and ORF4 partial
chn1	CP93-1309	China	GU327735.1	5 803	Complete genome
CHN-FJ4	FN02-3924	China	JQ266226.1	2 915	ORF1, ORF2 and ORF5 partial, ORF3 and ORF4 complete
CHN-GD3	YT86-368	China	JQ266228.1	2 876	ORF1, ORF2 and ORF5 partial, ORF3 and ORF4 complete
CHN-GD-GZ10-1	CP93-1634	China	HQ245324.1	1 284	ORF3 and ORF4 complete
CHN-GD-GZ10-3	CP93-1634	China	HQ245326.1	1 284	ORF3 and ORF4 complete
CHN-GD-ZC1	*Saccharum* spp.	China	HQ245328.1	1 284	ORF3 and ORF4 complete
CHN-GD-ZJ1-2	*Saccharum* spp.	China	HQ245330.1	1 284	ORF3 and OFR4 complete
ColMex-317	CP 72-2086	Mexico, Colima	KT334298.1	512	ORF3 partial
Haw1	H78-3606	USA, Hawaii	AJ491261.1	672	ORF5, ORF3 and ORF4 partial
Haw2	H87-4094	USA, Hawaii	AJ491262.1	672	ORF5, ORF3 and ORF4 partial
J55/487	*Saccharum* spp.	Kenia, Kilifi	KR608794.1	996	ORF3 complete
Kampala	*Saccharum* spp.	Kenia, Kilifi	KR608793.1	996	ORF3 complete
Maur1	M1658-78	Mauricio	AJ491263.1	672	ORF5, ORF3 and ORF4 partial
Maur2	M2350-79	Mauricio	AJ491264.1	672	ORF5, ORF3 and ORF4 partial
PER-YL1a	H50-7209	Peru	AM072752.1	5 612	Complete genome
PER-YL1b	H50-7209	Peru	AM072753.1	5 612	Complete genome
Reun2	R85-1102	Reunion	AJ491266.1	672	ORF5, ORF3 and ORF4 partial
REU-YL1a	R570	Reunion	AM072754.1	5 612	Complete genome
SA1	N30	South Africa	AJ491267.1	672	ORF5, ORF3 and ORF4 partial
SCYLV-C4	CC84-75	Colombia	AF369929.1	2 832	ORF1, ORF2 and ORF5 partial, ORF3 and ORF4 complete
ChT-11	NA	Mexico	AJ012650.1	924	Gen CP of PRSV

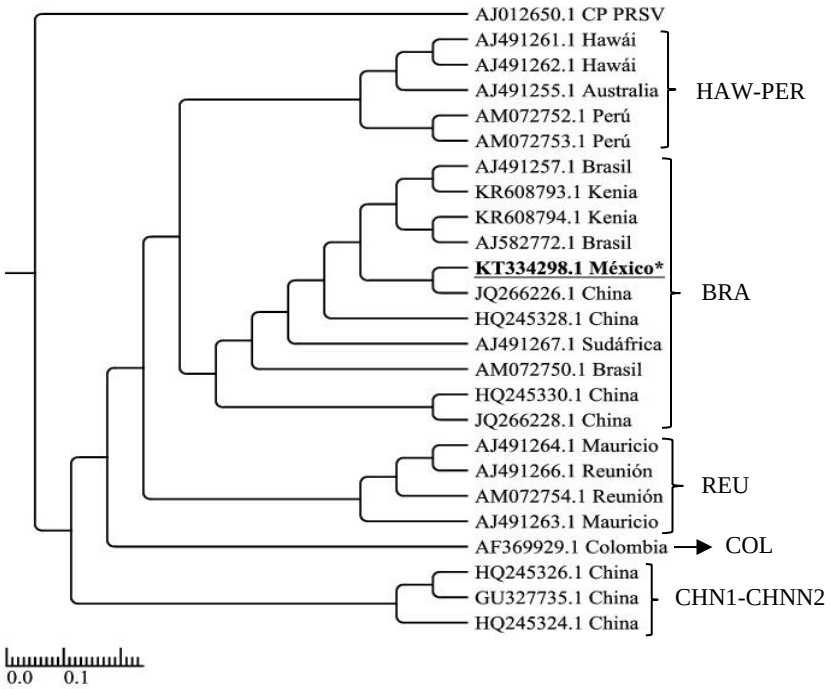

Figure 3. Phylogenetic tree based on the UPGMA method and maximum likelihood using partial sequences and complete genomes of SCYLV. The CP sequence of the PRSV (*Papaya ringspot virus*) was used as an external group and bootstrap analysis was performed with 1000 replicas. The Mexican isolate of this study is highlighted with an asterisk. Genotypes HAW-PER= Hawaii-Peru, BRA= Brazil, REU= Reunion, COL= Colombia and CHN= China.

In another study Chinnaraja *et al*. (2013) obtained similar groupings, in addition to other clusters corresponding to other genotypes reported for SCYLV. In this study, the same tendency of formation of three aforementioned main clusters was obtained, in addition to two other groups where the Colombian isolate was located and in another group sequences, of Chinese origin (Figure 3). The genetic variations between the alignments of the sequences that formed the phylogenetic tree were 0-18 nucleotides, the most diverse being the sequences of the CHN1 and CHN2 genotypes, which shared with the sequence ColMex317 (KT334298.1) percentages of identity of 96.29, 96.68 and 98.63% for CHN1 (HQ245326.1), CHN1 (GU327735.1) and CHN2 (HQ245324.1), respectively. The rest of the sequences had high identity percentages with ColMex317, greater than 99%. The sequence of Mexican origin was grouped with sequences from Brazil, China, Kenya and South Africa, so its origin is probably Brazilian since all the sequences of this group correspond to the BRA genotype however, it is necessary to analyze a greater number of sequences and of greater nucleotide length.

Several authors have reported an exclusive group for Reunion strains (REU genotype) (Wang *et al*., 2012; Chinnaraja *et al*., 2013; Amata *et al*., 2016; ElSayed *et al*., 2018). In this study, sequences from Reunion and Mauritius were grouped into a cluster, in which the two REU isolates that were homologous to each other shared 99.41 and 99.61% identity with two sequences from Mauritius: AJ491264.1 and AJ491263.1, respectively. However, given the low genetic variability and geographical proximity of these two islands (Reunion and Mauritius), it

is most likely the same REU genotype. The largest number of sequences considered in this work corresponded to the BRA genotype of SCYLV, which is the most abundant as observed in previous reports (Wang *et al*., 2012; Chinnaraja *et al*. 2013; Amata *et al*., 2016).

In China, phylogenetic analysis of 141 sequences from various parts of that country and the world showed that all SCYLV isolates were grouped into 8 genotypes, of which 107 isolates from China were classified into three genotypes (BRA, HAW, CHN3), where the BRA genotype was the most prevalent and was detected in all the sampled areas (102/107) (Lin *et al*., 2015). In Mauritius, Africa, SCYLV was identified and its genetic variants were determined. The genotype REU was detected in 10 varieties, while another 4 presented the genetic variant BRA-PER. A mixed infection of the CUB and REU genotypes was detected in the Co6304 variety, while the Q88 was coinfected by BRA-PER and REU genotypes (Joomun and Dookun, 2010).

In these works, specific oligonucleotides were used to discriminate between genetic variants of the SCYLV isolates; however, in this study these initiators were not used for this purpose. Therefore, it is necessary to carry out studies with the oligonucleotides used in the works described above to determine the genetic variants present in our country, taking into account isolates from the sugar cane regions of southeastern Mexico, which is where the sugarcane area is concentrated. more important. The results of this study help to understand the distribution of this virus in sugarcane crops and give a first approximation of the phylogenetic origin of SCYLV isolated from Mexico.

Conclusions

In the states of Colima, Jalisco and Nayarit, the presence of SCYLV, causal agent of the yellow leaf syndrome, was detected in the main sugarcane areas of several municipalities. This virus is widely distributed in the sugarcane areas of western Mexico, probably due to the propagation by infected stake seed and the presence of aphid vectors. The phylogenetic analysis where the Mexican isolate was included indicated that the virus has a Brazilian origin and corresponds to the BRA genotype since it was grouped with three isolates from that country, among others more from diverse geographical origins. In future works on the SCYLV in Mexico should be used longer nucleotide regions or taking into account that the genome of this virus is relatively small (approximately 5 800 nucleotides) can be used complete genomes to obtain more information about their genetic variability.

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