Geographical Distribution of Four *Sugarcane yellow leaf virus* Genotypes

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ABSTRACT

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Specific primer pairs were designed to distinguish four genotypes (BRA for Brazil, CUB for Cuba, PER for Peru, and REU for Réunion Island) of *Sugarcane yellow leaf virus* (SCYLV) by reverse transcription–polymerase chain reaction (RT-PCR). A unique genome fragment was amplified from each genotype, with the exception of genotypes BRA and PER that are phylogenetically relatively close and were designated genotype BRA-PER. These RT-PCR primers were then used to identify the SCYLV genotype(s) present in 18 different sugarcane growing locations in the world, and 245 leaf samples infected by the virus were analyzed. Most samples were infected by only one of the three genotypes, but mixed infections occurred. Genotype BRA-PER was found in all sugarcane growing locations, whereas genotypes CUB and REU were each found in four geographical locations only. Genotypes BRA-PER, CUB, and REU were all three detected in locally bred sugarcane cultivars in Guadeloupe, indicating local transmission of these genotypes. In contrast, only genotypes BRA-PER and CUB were found in locally bred cultivars in Brazil, whereas genotype REU was detected in this country in cultivar R570 imported from Réunion. Similarly, genotypes BRA-PER and REU are both present in Réunion, but genotype BRA-PER has not, as of yet, spread on this island. Presence of several SCYLV genotypes in Brazil, Colombia, Guadeloupe, Mauritius, and Réunion suggests different virus introductions and/or different evolution histories of the virus after its introduction into a new environment.

Additional keywords: *Saccharum* spp.

Sugarcane yellow leaf virus (SCYLV) is the causal agent of yellow leaf of sugarcane (*Saccharum* spp.), a disease previously called yellow leaf syndrome or YLS (28,29). Yellow leaf was first reported in Hawaii and in Brazil in the late 1980s and early 1990s (30,33), and it has since been reported in more than 30 sugarcane producing countries worldwide (4,20). The incidence of SCYLV in commercial fields can reach 100% in susceptible cultivars (6,7,25,34), and the disease can cause significant yield losses in susceptible cultivars even if infected plants do not exhibit overt disease symptoms (8,15,27,33).

A characteristic symptom of the disease is an intense yellowing of the leaf midrib on the abaxial surface of mature leaves,

and this discoloration often occurs while the lamina is still green. In some sugarcane cultivars, leaves show a red coloration of the midrib on the adaxial surface. The leaf blade can then become bleached, proceeding from the tip toward the base of the leaf. Dwarfing of terminal internodes and tissue necrosis can also eventually be observed. Infected plants show a reduction of sucrose in stalks and an increase of sucrose accumulation in midribs (10,13). Sometimes symptoms appear only when infected canes are stressed (17). However, most of these symptoms may be related to other biotic or abiotic factors such as stress conditions, insect damage, water logging, and cool winters (5,20). The virus resides in the phloem tissue of plants and is transmitted during vegetative propagation of sugarcane by planting infected cuttings (19,26,29,32). SCYLV is transmitted from plant to plant by the aphid vectors *Melanaphis sacchari*, *Rhopalosiphum maidis*, and *R. rubi-abdominalis* in a persistent, circulative, and nonreplicative manner (21,29,32).

SCYLV has recently been assigned to the genus *Polerovirus* of the family *Luteo-*

viridae (9), and several genetic diversity studies showed that SCYLV is a variable virus (1,2,22). Based on phylogenetic analyses of sequences of the entire translated genome of SCYLV, Abu Ahmad et al. (1) described the occurrence of three different genotypes (BRA, PER, and REU) within eight virus isolates from worldwide locations. The name given to each of these genotypes was based on the geographical location where it was first detected: Brazil, Peru, and Réunion, respectively. Sequence identity varied from 94.2 to 95.5% among isolates of genotypes BRA and REU, and from 93.9 to 94.1% among isolates of genotypes PER and REU. Although genotype PER isolates showed 96.7 to 98.5% sequence identity with genotype BRA isolates, genotype PER formed a separate lineage. Additionally, a virus isolate from Cuba that was partially sequenced showed only 77 to 80% amino acid sequence identity in open reading frame 1 (ORF1) with isolates of genotypes BRA, REU, and PER. This result suggested that the Cuban isolate represented another genotype or even an isolate of a new virus species (1). In the study herein, this virus isolate will be considered as another genotype of SCYLV and called CUB for Cuba.

The objective of our study was to analyze the distribution and incidence of SCYLV genotypes BRA, CUB, PER, and REU in different geographical locations in the world. We report here the design of specific primers to identify genotypes BRA (or PER), CUB, and REU by reverse transcription–polymerase chain reaction (RT-PCR), and the genotyping of SCYLV in 245 leaves infected by the virus and collected from 18 sugarcane growing locations from four continents (North and South America, Asia, and Africa).

MATERIALS AND METHODS

Infected plant material and RNA extraction. Leaves infected by SCYLV were collected from Cirad's sugarcane quarantine facility (3) or directly from diseased fields (Table 1). Tissue blot immunoassay (TBIA) and RT-PCR were used to verify the presence of SCYLV in leaf samples. TBIA was performed as described by

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Schenck et al. (31), except that Fast Blue BB salt (Sigma Chemical Co.) was used. RT-PCR with diagnostic primers YLS111 and YLS462 (1) and Ready-To-Go RT-PCR Beads (Amersham) was performed as described by Comstock et al. (4). The amplified fragment from the coat protein coding sequence has an expected size of 351 bp (fragment YLS, Fig. 1). Leaves were stored at -80°C until RNA extraction. Total RNA was extracted and purified from sugarcane leaves (100 mg of lamina and midrib) with the RNeasy Plant Mini Kit (Qiagen) using the manufacturer's protocol. Total RNA was eluted in a final volume of 40 μl of diethylpyrocarbonate-treated (DEPC) water and stored at -20°C .

SCYLV genome sequences and design of specific primer pairs for SCYLV genotyping. Sequences of the entire translated genome of 10 SCYLV isolates available in the GenBank data library (accession numbers AM072750, AM072751, AM072752, AM072753, AM072754, AM072755, AM072756, AF157029, AJ249447, AY236971) and sequences of an additional five SCYLV isolates (Y. Abu

Ahmad, M. Royer, and P. Rott, *unpublished data*) were used. Sequences were aligned and analyzed with DNAMAN Sequence Analysis Software (Lynnon Bio-Soft, Vaudreuil, Canada). A contiguous 5,609 to 5,612 bp (ATG ORF0 to stop codon ORF5) sequence was obtained for all 15 isolates. Genome regions that showed nucleotide variation among genotypes BRA, CUB, PER, and REU were used to design primers for RT-PCR amplification of a distinct fragment from each genotype. These primers were also tested in combination with nonselective primers that were previously described (1).

Amplification of a specific genome fragment from each SCYLV genotype by RT-PCR. A distinct fragment for each genotype was amplified by RT-PCR using the Titan One Tube RT-PCR System kit (Roche) and primer pairs that were located in different locations of the viral genome (Table 2 and Fig. 1). The same RNA template was used for all RT-PCR reactions performed with a given virus isolate. The 25- μl RT-PCR reaction mix consisted of 1 μl of eluted RNA, 5 μl of RT-PCR buffer

(5 $\times$), 1.25 μl of DTT solution (100 mM), 0.25 μl of RNase inhibitor (40 U μl^{-1}), 0.5 μl of dNTP mix (10 mM), 0.5 μl of Titan enzyme mix, 0.05 μl of each primer (100 μM), and DEPC water to final volume. The same RT-PCR program was used for all SCYLV genotypes: 56°C for 60 min, 94°C for 2 min, 30 cycles at 94°C for 15 s, 61°C for 2 min, and 68°C for 2 min with a final 68°C extension for 10 min. A 10- μl aliquot of each amplification product was analyzed through a 1.0% agarose gel by electrophoresis in TBE buffer, stained with ethidium bromide, and visualized under UV light. The amplification product of SCYLV genotypes BRA (or PER), CUB, and REU had expected sizes of 362, 450, and 905 bp, respectively (Table 2 and Fig. 2).

RESULTS

Selection of specific RT-PCR primers for identification of SCYLV genotypes. Several primer pairs were tested to distinguish SCYLV genotypes BRA, CUB, PER, and REU by selective amplification of a distinct genome fragment (data not

Table 1. Source of *Sugarcane yellow leaf virus* (SCYLV) infected leaves and identification of SCYLV genotypes in 245 samples from 18 different geographical locations

Origin of sample	Sugarcane host cultivar (interspecific <i>Saccharum</i> hybrids)	SCYLV genotype	Number of samples
Barbados	B85287, B86776, B9702, BT74209, BT7748, Co6806, DB8811, DB89138	BRA-PER	8
Brazil	R570, RB80-6043, RB84-5257, RB85-5113, SP71-1406, SP71-6163, SP77-5181, SP81-1763	BRA-PER ^a	15
Brazil	RB83-5054, SP71-6163, SP81-3250, SP83-5073	BRA-PER + CUB	4
Brazil	R570	BRA-PER + REU	2
China	CGT73-167, CMT70-611, CP70-1133, CYT93-159, CYT85-1589, CYZ81-173, FR93435, ROC3	BRA-PER ^a	10
Colombia	C1616-75, CC92-2227, CC92-2376, CC92-2885, Co421, CP38-34, Ja64-20, PR77-3007, SP71-6163	BRA-PER	16
Colombia	C1051-73, C236-51, C2655-74, CC84-75, CC85-96, CC87-505, CC92-2867, CC92-2882, CC92-2885, CC93-3811, CP38-34, Mex64-1487, POJ2878	CUB	21
Colombia	CC85-96	BRA-PER + CUB	7
Cuba	Ja64-11	BRA-PER	1
Cuba	C132-81	CUB	4
Florida	CP85-1491, CP86-1633, CP89-2143, R570, TCP87-3388	BRA-PER	9
Guadeloupe	B37-161, CP67-412, FR9453, SP79-1169	BRA-PER	11
Guadeloupe	B68409, FR9707, FR9976, FR99101, FR0020, FR0048, FR00102, FR00170, SP71-6163	CUB	13
Guadeloupe	CB56-171, FR90714, FR9707, FR9856, FR9979, FR9983, FR00306, R570, R578, SP71-3146, SP71-6163	REU	32
Guadeloupe	FR99273, SP71-6163	BRA-PER + REU	2
Guadeloupe	FR9604, SP71-6163	CUB + REU	2
Hawaii	R570	BRA-PER	5
Malaysia	TC2, TC4	BRA-PER	2
Martinique	B7623, B7656, FR91180, FR91485, FR91816	BRA-PER	5
Mauritius	M124/59, M253/58	BRA-PER	2
Mauritius	M1186/86, M2024/88, R570, SP71-6163	REU	6
Mauritius	R570	BRA-PER + REU	3
Peru	H32-8560, H50-7209	BRA-PER ^b	3
Philippines	VMC71-238, VMC76-16, VMC87-599	BRA-PER	3
Réunion Island	"SP71-6163" ^c	BRA-PER	1
Réunion Island	R490, R570, R577, R84408	REU	30
Réunion Island	"SP71-6163" ^c	BRA-PER + REU	15
Senegal	CP72-2086, CP85-1491, CP86-1664, NA6390	BRA-PER	5
Sri Lanka	SLC9225, SLC9272, SLC9273, SLC9294	BRA-PER	4
Sudan	KnB96207, KnB96223	BRA-PER	2
Taiwan	ROC6, ROC10	BRA-PER	2

^a Isolates from sugarcane cultivar SP71-6163 in Brazil and cultivar CGT63-167 in China belong to genotype BRA based on the phylogenetic study of the entire translated genome of SCYLV (1).

^b Isolates from sugarcane cultivar H50-7209 belong to genotype PER based on the phylogenetic study of the entire translated genome of SCYLV (1).

^c Misabeled cultivar.

Fig. 1. Genome organization of *Sugarcane yellow leaf virus* as described by Moonan et al. (23) and location of the fragments amplified by reverse transcription–polymerase chain reaction (RT-PCR). UTR = untranslated region, ORF = open reading frame.

shown). Virus isolates representative of each genotype were used, and at least one specific primer pair was successfully identified per genotype to differentiate the four genotypes, with the exception of genotypes BRA and PER, which are phylogenetically relatively close (Table 2). The primer pair designed and chosen for amplification of genotype BRA isolates also amplified the two known isolates of genotype PER from Peru. Genotype BRA-PER will therefore be used below to designate this group of two closely related genotypes.

Genotyping of SCYLV in 18 sugarcane growing locations. The SCYLV genotype(s) occurring in 245 infected leaf samples from 18 different sugarcane growing locations in the world was determined by RT-PCR amplification with three different primer pairs (Tables 1 and 2). Genotypes BRA-PER, CUB, and REU were found in 137 (56%), 51 (21%), and 82 (33%) of the 245 samples, respectively. Mixed infections of genotypes BRA-PER + REU, BRA-PER + CUB, and CUB + REU were detected in 22 (9%), 11 (4%), and 2 (1%) of the 245 samples, respectively (Fig. 2).

At least one leaf sample from each of the 18 investigated geographic locations was infected by genotype BRA-PER (Table 1). BRA-PER was the only genotype found in Barbados, China, Florida, Hawaii, Malaysia, Martinique, Peru, Philippines, Senegal, Sri Lanka, Sudan, and Taiwan. Genotype CUB was found only in Brazil (in 4 of 21 samples), Colombia (in 28 of 44 samples), Cuba (in 4 of 5 samples), and Guadeloupe (in 15 of 60 samples). Genotype REU was found only in Brazil (in 2 of 21 samples), Guadeloupe (36 of 60 samples), Mauritius (9 of 11 samples), and Réunion Island (45 of 46 samples).

Table 2. Sequence and position in the *Sugarcane yellow leaf virus* (SCYLV) genome of the primers used for genotype identification by RT-PCR

Amplified fragment ^a	Name of primers	Genome position ^b	5'-3' sequence ^c
BRA-PER	BRA-PER-F	1,905-1,924	AACTGCTGCgtcAgGCcCa
	BRA-PER-R	2,267-2,246	GACGAGCtTGCGTTGttttCt
CUB	CUB-F	423-443	gTGCttcTCCcgGCGGTTcact
	CUB-R	873-853	attcGaGAACAACCTcCgcctc
REU	REU-F	3,540-3,558	CAAgCTtCTAGCgGGaaTc
	B-REV ^d	4,445-4,427	CAGTTGCTCaATGCTCCAcG

^a See genome location in Figure 1.

^b Position in the SCYLV complete genome (GenBank accession no. AF157029).

^c Polymorphic nucleotides are written in lower case.

^d Primer previously described by Abu Ahmad et al. (1).

Locally bred varieties were all infected by genotype BRA-PER in Brazil (15 samples) and by genotype REU in Réunion Island (30 samples) (Table 3). Two to three genotypes were found in locally bred varieties in Colombia (BRA-PER and CUB), Guadeloupe (BRA-PER, CUB, and REU), and Mauritius (BRA-PER and REU). SCYLV genotype CUB showed the highest incidence in locally bred varieties in Colombia where 22 of 26 samples (84%) were infected by this genotype. In Guadeloupe, genotype REU was the most common, and 18 of 29 samples (62%) collected from locally bred varieties were infected by this genotype.

DISCUSSION

These three genotypes of SCYLV are not distributed uniformly in the world. Genotype BRA-PER was found in 18 sugarcane growing locations in Africa, Asia, and North, Central, and South America, whereas genotypes CUB and REU were found in only four locations each. World distribution of genotype BRA-PER suggests that yellow leaf was originally caused by this genotype, which was spread

worldwide by infected plant material when the causal agent of yellow leaf was unknown and not intercepted in sugarcane quarantines (3). Although genotype BRA-PER is present all around the world, its incidence varied according to the sugarcane growing location. It was found in all sampled sugarcane varieties in Barbados, Brazil, China, and Florida; but in Réunion Island, it was only detected in an imported foreign variety. This latter variety was initially thought to be SP71-6163, the variety severely affected by yellow leaf in Brazil (20,33), but recent genomic fingerprinting showed that this variety was mislabeled (J. Pauquet, *personal communication*). Several reasons may explain the absence of spread of genotype BRA-PER in Réunion Island. This SCYLV genotype may have only recently been imported to this location highly contaminated by SCYLV (25,27), and has not been significantly spread yet because of limited inoculum sources. It can also be hypothesized that insect vectors present in Réunion Island do not efficiently spread genotype BRA-PER or that the local environment is not favorable to genotype BRA-PER. A

Fig. 2. Reverse transcription–polymerase chain reaction (RT-PCR) amplification products of three genotypes of *Sugarcane yellow leaf virus* (SCYLV) with genotype-specific primers. Lanes: 1 = ladder (1 kb); 2 to 9 = sugarcane leaf samples infected by SCYLV isolates PER-YL1 from Peru, CUB-YL1 from Cuba, REU-YL1 from Réunion, REU-YL2 from Réunion, BRA-YL1 from Brazil, COL-YL1 from Colombia, CHN-YL1 from China, REU-YL3 from Réunion, respectively; and 10 = virus-free sugarcane leaf sample. Each virus isolate was a single genotype with the exception of REU-YL3, which was a mixed infection (genotypes REU + BRA-PER).

Table 3. Genotypes of *Sugarcane yellow leaf virus* (SCYLV) in locally bred and imported varieties in five sugarcane growing locations

Location	Variety type (total number of varieties)	Number of samples infected by SCYLV genotype(s)						Total number of samples
		BRA-PER	CUB	REU	BRA-PER + CUB	BRA-PER + REU	REU + CUB	
Brazil	Locally bred RB and SP (10)	11	0	0	4	0	0	15
	Imported varieties (1)	4	0	0	0	2	0	6
Colombia	Locally bred CC (9)	4	15	0	7	0	0	26
	Imported varieties (11)	12	6	0	0	0	0	18
Guadeloupe	Locally bred FR (16)	3	8	14	0	2	2	29
	Imported varieties (12)	8	5	18	0	0	0	31
Mauritius	Locally bred M (4)	2	0	2	0	0	0	4
	Imported varieties (2)	0	0	4	0	3	0	7
Réunion Island	Locally bred R (4)	0	0	30	0	0	0	30
	Imported varieties (1)	1	0	0	0	15	0	16

similar situation occurs in Brazil where genotype REU was detected in variety R570 imported from Réunion Island, but not in locally bred varieties. Strain-specific transmission has been reported for other viruses of the *Luteoviridae* family such as *Barley yellow dwarf virus* (BYDV) (12,14,24,35) and *Potato leafroll virus* (PLRV) (18). In contrast, in Colombia and Guadeloupe, two (BRA-PER and CUB) and three (BRA-PER, CUB, and REU) genotypes were detected in locally bred sugarcane varieties, respectively, which proved local transmission of all these genotypes because no diseases are known to be transmitted through the caryopsis of the sugarcane fruit (11). Local transmission of SCYLV genotypes BRA-PER, CUB, and REU in Guadeloupe was also demonstrated by the detection of these genotypes in sugarcane fields several months after planting of healthy tissue-cultured plants (data not shown). Several genotypes of SCYLV can therefore coexist in a geographical location, but also in a sugarcane plant as demonstrated by the detection of mixed genotype infections in a few samples.

Genotype CUB was only found in South America (Brazil, Colombia, and Cuba) and in Guadeloupe. Genotype REU was only found in locally bred varieties in Guadeloupe, Mauritius, and Réunion Island. The limited geographical distribution of these two genotypes might be due to specific

pathogen × host × vector × environment interactions, and therefore different evolution histories. Future surveys and genotyping studies in the geographical locations sampled in this study should supply useful data regarding evolution of SCYLV populations.

Because *Saccharum* species (including traditional and modern sugarcane cultivars and wild relatives) are the only known natural hosts of SCYLV (19,20,32), and because these species (with the exception of some *S. spontaneum* clones in part of Africa) are not native to the Americas, Africa, or the Mascareines Archipelago (16), SCYLV was imported into these locations from elsewhere via infected planting material. The presence of several genotypes of SCYLV in Brazil, Colombia, Guadeloupe, Mauritius, and Réunion Island suggests different virus introductions. Additionally, other genotypes of SCYLV not yet described may occur in the world, especially in traditional sugarcane cultivars (*S. officinarum*, *S. barberi*, *S. sinense*) and wild relatives (*S. spontaneum* and *S. robustum*) not tested in this study, and the biological significance of this genetic diversity remains to be determined.

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